Biodegradable polyesters for veterinary drug delivery systems: Characterization, in vitro degradation and release behavior of Oligolactides and Polytartrate

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“Wir stehen immer noch vor der Tür,
hinter der die großen Antworten warten.”

(Arthur Miller)

List of Publikations

Abstracts

- G. Schliecker, S. Fuchs, C. Schmidt and T. Kissel, Modified drug release from polyester implants: Polytartrate vs. coated PLGA implants. Proceed. 4th World Meeting ADRITELF/APGI/APV, Florence (2002).

- G. Schliecker, S. Fuchs, C. Schmidt and T. Kissel, Polytartrate- a less known class of biodegradable polyester, Proceed. Int. Symp. Control. Rel. Bioact. Mater. 30 Glasgow (2003).

Research Articles

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- G. Schliecker, S. Fuchs, C. Schmidt and T. Kissel, Synthesis and characterization of D,L-lactic acid oligomers: a mechanistic study to analyze the degradation kinetics in vitro, Biomaterials 24 (2003), 3835-3844.

- G. Schliecker, S. Fuchs, C. Schmidt, R. Wombacher and T. Kissel, Hydrolytic degradation of PLGA films: the role of oligomers on degradation rate on crystallinity, Int. J. Pharm 266 (2003), 39-49.

- G. Schliecker, S. Fuchs, C. Schmidt, A. Ehinger, J. Sandow and T. Kissel, In vitro and in vivo correlation of Buserelin release from biodegradable implants using statistical moment analysis, J. Control. Release 94 (1) (2004), 25-37.

- G. Schliecker, S. Fuchs, C. Schmidt and T. Kissel, Characterization and in vitro degradation of polytartrates, submitted to J. Control. Release.

Patent

- C. Schmidt, G. Schliecker, S. Fuchs, T. Kissel, Pulsatile release implants, 16.01.2002, EP 02075176.4

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Chapter 1

Biodegradable polymers and their potential use in parenteral veterinary drug delivery systems

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1. Introduction

Drug delivery plays an important role in the development of pharmaceutical dosage forms for the animal health care industry because often the duration of drug release needs to be extended over days up to several months. This can be achieved by incorporation of drugs into polymeric materials to control drug release at a predefined and reproducible rate for a prolonged period of time. The majority of veterinary drug delivery systems are fabricated from non-degradable polymers such as silicone, polyurethane and ethylene vinylacetate copolymers, which are inexpensive, biocompatible, biological inert and have received regulatory approval [1]. In recent years the interest for biodegradable polymers as veterinary drug delivery systems, which control and prolong the action of therapeutic agents, has grown in importance. The reason being that delivery systems based on biodegradable polymers do not require removal from the animals at the end of the treatment period due to their degradation into physiologically occurring compounds that can be readily excreted from the body. This provides significant benefits such as reduction of animal stress resulting from animal handling and physical removal of the delivery system, reduction of cost in terms of both finances and time spent by the end-user.

In veterinary medicine it is important to know whether the drug release system is indented for treatment of livestock or for companion animals, which are the two major categories of the animal health market. Livestock animals comprise primarily cattle, sheep, goats, swine and poultry but also fish and any other animals which enter the food chain [2].

Livestock industry compares treatment costs with benefits resulting from therapy thus the price of the medicament has to be as low as possible to allow profitable management for the farmer. On the other hand every visit of a veterinarian is associated with costs for the farmer and thus a biodegradable delivery system, which requires only a one-time application coupled with increased therapeutic effect, will be of economic benefit although the cost of such delivery system may be higher than conventional treatment.

The livestock products dominate the animal health market and account for approximately 70 % of total sales. The remaining 30% are attributed to companion animal products [3]. Companion animals or pets, such as dogs, cats and horses constitute the largest segment. Other animals such as birds, reptiles and rabbits can

also be considered as companion animals, however, these species are sometimes classified as exotic animals, which represent only a small fraction in the companion animal market [4]. The companion animal market is quite different from the livestock animal market. For one, the number of animals eligible for treatment is small and the outlay is directed toward a single animal. Secondly, companion animals are often considered as part of the family and the arbitrary value of the animal for the owner allows premium veterinary care. Thus this segment of the animal health market presents opportunities for research synergies and spin-offs from human health with less consumer safety orietated regulatory pressure than the livestock animal market [5, 6]. Although human and animal health care industries show many similarities, the diversity of species and breeds, the range in body size, regional differences, differences in the biotransformation rate and other factors make the development of veterinary drug delivery systems more complicated [2]. Furthermore, additional regulatory requirements, particularly for food producing animals do exist. Because these animals enter the food chain tissue residues must be addressed for both the drug and the polymer. Thus residual levels of drug in tissue play an important role as major consumer safety issue and are the basis for withdrawal times, which determines the earliest time point after administration for slaughter. In the companion animal market the owner convenience is responsible for the product acceptance. Although injections are common and preferred for livestock animals, oral administration is preferred for companion animals. It should be noted, that it is very challenging for the pet-owner to administer tablets to the animal, especially to cats, if taste or odor are repulsive to them. Thus free choice acceptance of an oral dosage form is important for product acceptance. However, in many cases parenteral application is required to achieve sufficient therapeutic effect. Thus in companion animal medicine it can also be beneficial to formulate a drug, e.g. peptides or proteins into a biodegradable delivery system. This would allow to control animal fertility or to treat diseases like cancer in an advanced manner, which would improve both patient compliance and owner convenience.

In recent years biodegradable veterinary drug delivery systems such as microspheres, implants and in-situ forming implants have been tested in the area of estrus control [7], growth promotion [5], control of ectoparasites [8] and vaccine delivery [9].

Biodegradable polymers, which allow delivery of a range of bioactive materials with high bioavailability, have demonstrated their potential for veterinary application.

However, presently only few biodegradable drug delivery systems are commercially available for veterinary use. Among other reasons, the final price of the device followed by regulatory considerations and challenges in formulation stability have limited the development of such delivery devices.

It is the intention of this chapter to give an overview of biodegradable polymers, which are used or tested in the veterinary field. The paper will highlight some recent developments in this area and will look into the future to examine the directions in which veterinary pharmaceutics is heading. Examples of currently available and future biodegradable veterinary drug delivery systems will be presented and explained including intravaginal devices, injectables and implantable systems.

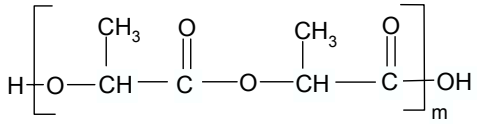
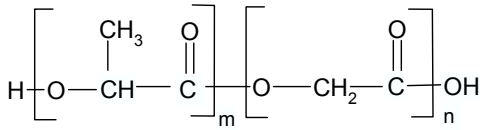
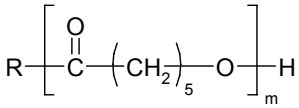
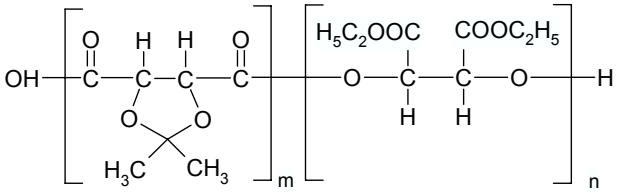
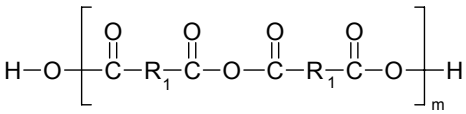
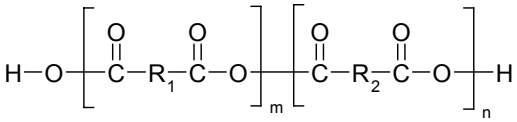
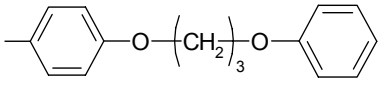
2. Biodegradable polymers for veterinary applications

The most attractive and commonly used biodegradable polymers are polyesters such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone (PCL) (Table 1). These materials are commercially available in different compositions and molecular weights which allows control degradation of the polymer [10, 11].

The term degradation designates the process of polymer chain cleavage which leads to a loss of molecular weight. Degradation induces the subsequent erosion of the material which is defined as mass loss of material ocess of polymer chain cleavage [12].

For degradable polymers two different erosion mechanisms have been proposed: homogeneous or bulk erosion, and heterogeneous or surface erosion [13]. The difference is illustrated in Fig. 1. Bulk-eroding polymers degrade all over their cross-section because the penetration of water into the polymer bulk is faster than degradation of polymer. In surface-eroding polymers, in contrast, degradation is faster than the penetration of water into the bulk. In consequence these polymers erodes mainly from its surface. However, for most polymers, erosion has features of both mechanisms. The erosion mechanism has consequences for the mechanism of drug release which has been classified into diffusion-, swelling- and erosion controlled.

Table 1 Chemical structures of biodegradable polymers

Type	General structure	Example
Polyester		Poly(lactide)
		Poly(lactide-co-glycolide)
		Poly(ε-caprolactone)
		Polytartrate
Poly-anhydride		Poly(sebacic acid) $R_1 = –(CH_2)_8$ Poly(fumaric acid) $R_1 = –CH=CH$
		Poly[1,3-bis-(p-carboxyphenoxy)propane-co-sebacide] $R_2 = –(CH_2)_8$ $R_1 =$ 

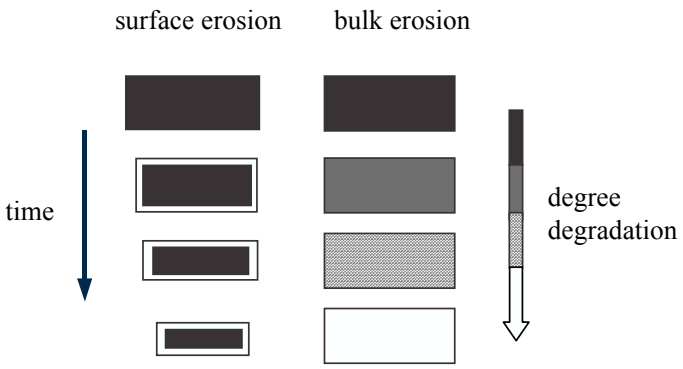


Figure 1 Schematic illustration of surface erosion and bulk erosion

A biodegradable polymer device might release the drug by all three mechanisms and the fastest mechanism dominates (Fig. 2). In case of biodegradable polyesters, which consist of monomers connected to each other by ester bonds, degradation starts after penetration of water into the device. The breakage of ester bonds occurs randomly via hydrolytic ester cleavage and leads to the subsequent erosion of the device. The hydrolysis rate is influenced by molecular weight, copolymer ratio, polydispersity and crystallinity, which can be used to control drug release. For example, poly(ε-caprolactone) which is a high hydrophobic and crystalline polyester degrades very slowly compared to amorphous less hydrophobic PLGA. Depending on these variables the degradation time varies from several weeks up to years and allows the release of drugs over this time period. However, to achieve controlled drug release from polyester based delivery systems is difficult because these polymers undergo bulk erosion which changes the polymer matrix and influences drug release. As a consequence, drug release is controlled by swelling, drug diffusion and polymer erosion, which is not straight forward to predict [10, 14, 15].

The above mentioned polyesters have one characteristic in common: the hydrolytic sensitive groups are located in the polymer backbone. This feature stimulated the development of a new class of biodegradable polymers based on tartaric acid which contain additionally to ester bonds in the backbone, ester as well as ketal bonds in the polymer side chains [16] (Table 1). These, so called “polytartrates” seem to be promising due to the modification in the polymer structur. Early experiments demonstrated the suitability of described polytartrates for controlled release applications [17].

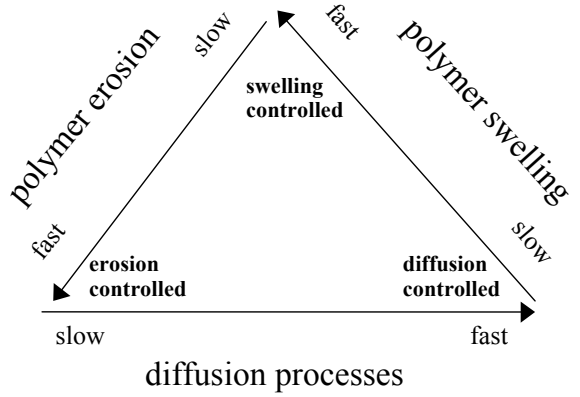


Figure 2 Possible mechanism of drug release from degradable polymers

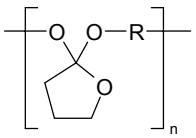
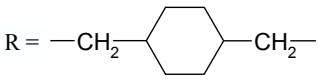
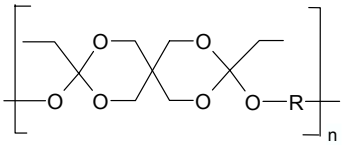
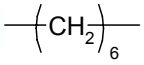
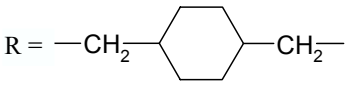
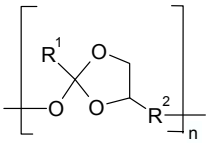
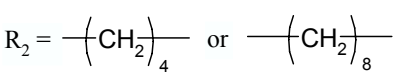
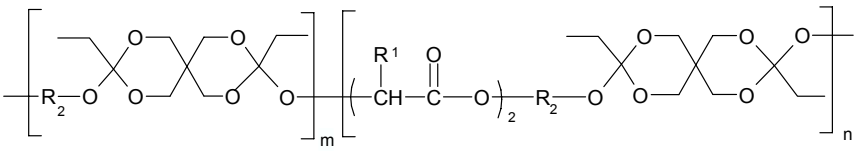
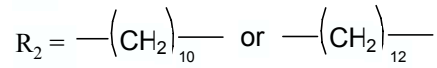
However, until now these polytartrates have not received commercial status and only few information is available about this polymer class [18].

Due to the fact that in general polyesters degrade over a longer time period, which is disadvantageous when the drug needs to be released for only few days or weeks the interest was shifted to polymers, which degrade faster and allow control of drug release exclusively by polymer erosion. This leads to the development of poly(ortho esters) (POE) and since the late 1970s, four families of POE were developed (Table 2). The synthesis of POE and their use in controlled drug release has recently been reviewed [19].

The polymers of the first (POE I) and second (POE II) generation of POE are solid materials whereas the polymers of the following generations (POE III, IV) are semi-solids. They allow the incorporation of therapeutic agents or additives by simple mixing, avoiding the need for solvents or elevated temperatures which is a significant advantage over other biodegradable polymers. Because ortho ester linkages are acid-labile, degradation rate can be modulated by pH. Lowering the pH at the polymer-water interface accelerates the hydrolysis rate whereas an increase of pH results in a lower hydrolysis rate [20]. In POE IV the degradation rate of ortho ester bonds is controlled by lactic acid units which are generated by cleavage of the polymer backbone. The degradation time of POE can vary from few days to several months and therefore these polymers are of interest for short- and long acting delivery systems [21, 22].

Another class of biodegradable polymers are polyanhydrides which were investigated for drug delivery systems in the early 1980s and their number

Table 2 Chemical structures of four families of poly(ortho esters)

Type	General structure of poly(ortho esters)	
POE I		R = –$(CH_2)_6$– 
POE II		R =  or 
POE III		R_1 = $-CH_3$ 
POE IV		R_1 = —H or $-CH_3$ and 

increased tremendously in the recent years [12, 23]. The best characterized polyanhydrides are p(CPP-SA) and p(FA-SA). The first is a copolymer of sebacic acid (SA) and 1,3-bis(p-carboxyphenoxy)propane (CPP) whereas the second is a copolymer of sebacic and fumaric acid (FA) (Table 1). The advantage of polyanhydrides can be seen in the fact that they contain the most reactive functional group available for degradation. Based on their chemical nature polyanhydrides degrade very fast at a predictable rate for periods of few days up to weeks [23]. Thus the main application of this polymer class is in short-term controlled delivery of bioactive agents. The degradation rate can be adjusted by hydrophobic and

hydrophilic components in the copolymer. In contrast to PLA, PLGA and PCL, polyanhydrides and POE are thought to undergo surface erosion since they are assembled from fast degrading functional groups. Therefore drug release should be controlled mainly by polymer erosion. However it seems that surface erosion is a characteristic that is strongly linked to the dimension of a device and that, below a critical size limit, this property is lost [24].

3. Therapeutic applications of biodegradable polymers

3.1 Biodegradable delivery systems for the control of animal reproduction

There are two different areas of animal reproduction control: estrus synchronization and contraception. Whereas the first is very important for livestock production and breeding the latter is of special interest in companion animals.

In livestock animals deficient estrus detection is in many cases responsible for infertility and therefore the major reason for manipulating the estrus cycle. The difficulties in estrus detection based among other things on the short period of sexual receptivity where ovulation and insemination can take place. Therefore, attempts have been made to synchronize estrus to make female animals fertilizable at a predetermined time schedule. This has several advantages for the farmer as well as the breeder:

- reduced time and labor currently devoted to estrus detection and to allow more cost effective implementation of timed insemination programs
- increased use of artificial insemination with fresh, transported or frozen semen to enhence genetic breeding variability and prevent communicable diseases resulting from natural service
- synchronize donor and recipients for embryo transfer allowing the use of new assisted reproductive technologies such as superovulation, in-vitro fertilization or cloning
- allows breeding throughout the year and
- improving reproductive efficiency and hence farming [2].

Poly(ε-caprolactone) (PCL) has been shown to be suitable for manufacturing of an intravaginal drug delivery system for the delivery of progesterone for estrus cycle control and synchronization in cows [25, 26] and sheeps [27]. Cattle were treated for 7 days either with a PCL insert or the commercially available, non-degradable silicone

insert (CIDR-B™) both containing 10% w/w progesterone. The PCL insert achieved similar average progesterone plasma concentrations compared to the silicone insert over the 7 days insertion period [25].

The incorporation of excipients such as lactose, polyethylene oxide or various types of cyclodextrin can be used to modify progesterone release from the intravaginal insert [26]. Anestrus sheeps, which were treated with a progesterone-containing PCL insert, showed elevated plasma progesterone levels similar to those obtained from the commercial silicone insert (CIDR-G™) over the 14 days insertion period [27].

Recently, a more versatile PCL intravaginal insert was developed which allows the incorporation of a large number of drugs [28]. This was achieved by using both extrusion and injection molding technique. Progesterone and PCL were compounded by extrusion to small pellets which were then injection molded into inserts. Additionally, the surface area of the insert was modified by cutting off certain sections of formed insert and replacing with blank PCL. Ovariectomized cows were administered either the optimized PCL insert or a commercial silicone insert (CIDR-B™). To achieve the same plasma progesterone levels as the CIDR insert the surface area of the PCL insert was increased. It was found that the surface area is the only significant variable, which effects plasma progesterone concentration. The pharmacokinetic values over a 7-day insertion period suggest that the optimized PCL insert is bioequivalent to the commercial silicon insert. The PCL insert was well tolerated by the animals and field studies have shown that the PCL insert containing 10 % w/w progesterone to be clinically at least as effective as the commercial product.

In addition to intravaginal inserts biodegradable microspheres were investigated to control estrus and ovulation in horses [29, 30], pigs [31], and cattle [32].

In general, poly(D,L-lactide) has been used to produce steroid containing microspheres by a solvent extraction process and currently one formulation is commercial available for accurate control of ovulation in mares (Lutamate Plus™).

The microspheres for intramuscular injection (i.m.) contain 100 mg estradiol and 1250 mg progesterone. In a controlled multicentered clinical trial using 135 mares the proportion of mares displaying a normal estrus following treatment increased from 63 to 87%. The variation for days in estrus decreased which demonstrated the clinical efficacy of this product [29]. In a separate study DL-PLA microspheres containing 100 mg estradiol were also successfully tested in horses [30]. The administration of this formulation to pigs resulted in pseudopregnancy for greater than 50 days. This

was successfully used to induce estrus on day 59 by application of a commercial PGF2α product [31]. For estrus control in cattle a combination of two different progesterone containing DL-PLA microspheres was investigated. The observed plasma progesterone levels were similar to those observed following intravaginal administration of a commercial available progesterone insert [32].

To achieve fertility control in pets various biodegradable delivery systems were investigated for the application of gonadotropin-releasing hormone (GnRH) analogs. PLGA is the most frequently studied polymer in this field and microparticles [33], extruded implants [34, 35] or in-situ formed implants [36, 37] prepared from various types of these polymers containing GnRH analogs were successfully used for chemical castration in dogs for 1 to 6 month.

Other polymers that were investigated for reproduction control in veterinary field are polyanhydrides. To induce ovulation and spermiation in fish p(FAD-SA) microspheres containing a gonadotropin-releasing hormone (GnRH) analog (D-Ala^6, Pro^{9}NEt-GnRH) were prepared by a double emulsion technique [38]. Two commercial important fishes, the striped bass and the Atlantic salmon were treated. All female fishes ovulate either within 11 days (Stripted bass) or 15 days (Atlantic salmon) after microsphere administration and were also effective in enhancing sperm production in male fishes.

However, scaling up commercial microspheres production to meet the demands of animal market is a very complex process which required costly facilities, water systems and equipment [5].

A more cost effective delivery system compared to microspheres are implants formed in-situ. The technology based on the fact that biodegradable polymers like PLGA spontaneously form solid depots when a solution of the polymer is injected into water. First, the polymer is dissolved in a pharmaceutically acceptable solvent such as N-methyl-2-pyrrolidone (NMP) or benzoyl benzoate. Thereafter the solution is mixed with the active pharmaceutical ingredient and the resulting solution or suspension can be easily injected either subcutaneously or intramuscularly using a small gauge needle [7, 39].

After injection displacement of the carrier with water in the tissue fluids causes the polymer to precipitate to form a solid film or implant (Atrigel technology™). The drug encapsulated within the implant is then released in a controlled manner as the polymer matrix biodegrades with time. The timeframe of the release can be adjusted

using different formulation variables, chiefly by altering the polymer composition and molecular weight [40]. The Atrigel technology™ was recently investigated for the controlled release of leuprolide in rats and dogs [36, 37]. Serum testosterone and leuprolide levels showed no significant difference in the pharmacologic efficacy compared to marketed leuprolide-containing microspheres (Lupron Depot™). Due to the simple manufacturing technique this technology is more cost effective than marketed microspheres and implant products and appears promising for product development. However, NMP which is often used as solvent for PLGA causes pain reactions during the application and therefore alternative solvents would be beneficial for veterinary use [7].

Viscous poly(ortho esters) allow subcutaneous injection and avoid the need for organic solvents. Recently, a low molecular weight POE, containing 30 % of lactic acid units in the polymer backbone (POE70LA30) was used for estrus synchronization in sheep [41]. Fluorogestone acetate (FGA), a potent synthetic progestagen, which is used in several non-degradable intravaginal inserts or sponges, was added to POE70LA30 (1.5 and 3 % w/w) by mixing. The addition of 20 % poly(ethylene glycol) increased the syringeability of the formulation and the cumulative release. Nevertheless, FGA was released slowly and almost constantly and only 29 % of incorporated FGA was released in vitro after 14 days. In vivo testing in sheep is currently in progress to determine the efficacy of these POE-based formulation.

3.2 Biodegradable delivery systems for the control of ectoparasites

The control of ectoparasites such as fleas, flies, ticks and mites is of great importance in the animal health market. In livestock animals infections with ectoparasites leads to animal suffering and hence to e.g. weight loss and reduced milk production which effects finally the productivity. In companion animals ectoparasites causes e.g. skin diseases which affect the well-being of the animals. The research and development costs for the discovery of new chemical entities to control parasites in both lifestock and companion animals have increased significantly. In parallel advances have been made in the development of biodegradable drug delivery systems [42]. For such delivery systems drug substances which are highly efficient at extremely low dosages are ideal candidates such as ivermectin, a semi-synthetic macrocyclic lactone [43].

Ivermectin containing microspheres were obtained by a solvent evaporation technique using PLGA, copolymer ratio 50:50 and 90:10, as well as PLA as matrix [44]. The three formulations were tested in Spanish goats and ivermectin was released at therapeutic levels about 10-12 weeks from PLGA microspheres or in combination with PLA microspheres released for at least 24 weeks. The PLGA 50:50 formulation controlled the ticks for 12 weeks after treatment. In addition, the treatment provided inhibition of larval horn flies in the manure of treated animals for 10 weeks.

The efficacy of injectable microspheres containing 30 % ivermectin for control of cattle fever ticks was tested using a blend of PLGA microspheres (half 50:50 copolymer and half 65:35 copolymer) [45]. No engorged ticks were found 4 weeks after treatment until the end of the study at week 16. The treatment eliminated also the tick population in the pasture where the cattle were held. In addition, treated cattle gained an average of 35 kg more than untreated animals [46].

The application of PLGA and PLA yielded to a long-term delivery system for ivermectin, which needs to be administered less frequently than commercial bolus systems (IVOMEC® SR). Furthermore, the total quantity of drug needed to control ectoparasites is reduced when using microparticles instead of an intraruminal bolus system.

Recently a product based on a biodegradable polymer for the prevention of canine heartworm in dogs was approved and is available in Australia, the USA (ProHeart™) [47] and Italy (Guardian SR Injectabile™) [48].

Moxidectin, an ivermectin derivative was incorporated into PLGA microspheres and protection against heartworm infection was achieved over a period of 6 months [49, 50] up to 1 year [48].

Poly(D,L-lactide) and PLGA were also tested for control of cattle grubs using methoprene, a juvenile hormone mimic, which was formulated into implantable pellets or microspheres [51]. When injected subcutaneously in the ears of infested cattle the formulations prevented the emergence of adult cattle grubs. Polycaprolactone was also investigated as release agent for methoprene and insect steroid analogues against ticks [52, 53].

Another polymer that has been tested for control of ectoparasites is POE. Ivermectin was covalent bonded to the POE monomers during synthesis and the resulting crosslinked polymer mass was finally extruded to a rod. The rod, which was indicated for control of heartworm in dogs, released ivermectin for as long as 6 months [54].

3.3 Biodegradable delivery systems for vaccination

Prevention of infectious diseases is a primary concern of animal health. Infectious diseases cause economic losses for livestock producers due to the decreased productivity. On the other side, without a good vaccination program, companion animals would suffer many serious infections. To prevent loss of animals vaccination is the most successful procedure.

A good vaccine delivery system is characterized by a controlled release of antigen in a pulsatile manner over a long time period to eliminate or reduce the need of subsequent inoculation and achieves a very effective protection against the intended disease.

Biodegradable microspheres have been widely investigated for vaccine delivery [55, 56, 57, 58]. In literature are also many reports documenting the great potential of biodegradable polymers for the prophylactic control of veterinary pathogens but until now there are no commercially available veterinary vaccines. Some examples for vaccine delivery using biodegradable polymers that have been evaluated in veterinary medicine are given in Table 3.

Copolymers of polylactide and polyglycolide esters have been widely used to produce biodegradable microspheres that act as depot for vaccine antigens [59, 60]. Microspheres less than 10 μm in diameter have been reported to be phagocytosed by macrophages whereas larger particles have to breakdown in vivo before they can be phagocytosed. This was demonstrated by injecting mice intraperitoneally staphylococcal enterotoxin B toxoid containing PLGA microspheres of 1-10μm and 20-125μm in diameter or a mixture of both [59]. Thus administration of a microspheres mixture, which differs in particle size, can induce long lasting immunity. This can also be achieved by using microparticles of different composition and molecular weight. The concentration of antigen affects also the rate of antigen release and subsequent induction of immune response. The higher the

Table 3 Biodegradable delivery systems for vaccination

Polymer	Delivery system		Virus/ Bacteria	Target animal
	Microspheres	In-situ implant		
PLGA	[59]		Staphylococcus	
		[60]	Ovalbumin antigen	Mice, swine
		[60]	Inactivated Pseudoarabies virus (PRV)	Swine
		[60]	Parvovirus	Canine
	[61]		Venezuelan equine encephalities (VEE) virus	Horse
	[62]		Parainfluenza-3 virus	Cattle
	[63]		Salmonella enteritidis	Poultry
	[64]		Fascioloa gigantica	Cattle
	[66]		Rabies virus	
	[67]		Salmonella thyphimurium	
	[68, 69]		Tetanus toxoid	

antigen loading the faster the release due to the presence of more antigen near the surface [60].

There are many viral infections of animals that require vaccines to induce antibodies. However, in many cases cell mediated immunity is necessary or beneficial to prevent diseases. Inactivated Venezuelan encephalitis virus (VEE) has been encapsulated in PLGA microspheres was injected to mice. Mice, which were vaccinated with microspheres, were better protected than mice treated with unencapsulated virus. Such a vaccine may be useful to prevent encephalitis for e.g. horses [61]. An example for encapsulation of a viral vaccine into PLGA microspheres is the parainfluenza-3 virus. This virus is part of the respiratory disease complex that causes high economic losses in beef cattle in North America. Cattle, which were treated with PLGA microspheres containing parainfluenza-3 virus, showed high antibody titers for up to 70 days [62]. In another veterinary example

PLGA was encapsulated with Salmonella enteritidis, a bacterial vaccine [63]. The microspheres were administered to hens for preventing infection in chickens by passive immunity in the yolk. The tested formulation was effective in stimulating the immune system for 9 month.

The encapsulation of Fasciola gigantica subunit antigen into PLGA microspheres demonstrated the feasibility of PLGA microspheres for delivering subunit antigens from intestinal/ systemic parasites of veterinary importance [64]. Fasciola hepatica is a worm that damages the liver and causes the disease liver fluke. The disease primarily affects cattle and sheep but also horses; deer, goats, pigs and dogs. This disease costs farmers over US$ 25 Million, yearly [65] with deaths and lost production, due to reduced weight gains, milk production and fertility. These very serious losses could be greatly reduced by the treatment of animals with a suitable vaccine formulation.

As described previously, PLGA and their homopolymers are suitable polymers for the in-situ implant technology. In a pilot experiment the Atrigel™ formulation, containing ovalbumin (OVA) as antigen was tested in mice and swine. It could be shown, that the in-situ implant effectively immunized swine to produce IgG response against small amounts of enclosed OVA after a single administration. Subsequent experiments demonstrated that the Atrigel™ technology is also suitable for delivering of complex antigens such as inactivated pseudorabies virus (PRV) vaccines to swine [60].

3.4 Biodegradable delivery systems for growth promotion

Growth promoting implants using steroids have been used for over 40 years in livestock production because they improve growth rate (+10 to 30%), feed efficiency (+5 to 15%) and carcass leanness (+5 to 8%) [70].

A continuing goal of livestock industry is to increase the quantity of high-quality lean tissue. The development of recombinant technology allowed the large-scale production of somatotropin and their commercial use to increases lean content and to reduce fat content of meat. Somatotropin increases also the milk production in dairy cows. However, somatotropin as well as other peptides and proteins loose on activity when dosed orally and the absorption via this route is poor due to their physicochemical properties [71]. Thus parenteral delivery systems for sustained

release of growth promoting peptides and/or proteins such as somatotropin, growth hormone releasing factor (GHRF) analogs or synthetic growth hormone releasing hormones (GHRH) are continuously under development in veterinary medicine.

Biodegradable polymers, especially in the form of injectable microspheres have been investigated for their capability of releasing growth promoting drug substances to livestock animals [71, 72, 73, 74]. Polyglycolic acid was used to encapsulate porcine somatotropin (pST) into microspheres. However, an incomplete release (less than 30% of drug loading) was observed, which was assigned to the instability of pST within this formulation [73]. This resulted in the development of more stable peptides and proteins, which stimulate the release of somatotropin, such as GHRF analogs and rismorelin porcine, a synthetic GHRH. Poly(lactide-co-glycolide), copolymer ratio 85:15, and rismorelin porcine were formulated into microspheres using a modified solvent evaporation process, which reduces the water solubility of the peptide and decreased loss of peptide during process [74]. Administration of rismorelin porcine-containing microspheres to pigs leaded to reduce excretion of urea nitrogen in urine and serum, which indicated that pigs converted urea into protein and muscle as response of treatment. Rismorelin porcine was delivered at a consistent rate over an extended period of time, which demonstrated that PLGA microspheres are suitable for long term delivery of this peptide. It should be noted that the duration of activity depends among other things on the suspension vehicle, which is used for microspheres injection. Another approach to enhance growth performance was the administration of PLGA microspheres containing a GHRF analogue to cattle [72]. Released GHRF analogue caused an increase in serum somatotropin concentration over 2 weeks and future studies are necessary to determine which serum ST concentration is sufficient for growth promoting.

Currently there is no biodegradable formulation for growth promotion on the market. A reason therefore is the high cost which is necessary to insure product quality and consistency. A delivery device based on PLGA for the long-term delivery of monensin sodium, an antimicrobial agent to promote growth promotion in cattle, is no longer commercial available (Monensin RDD™)[75]

The use of growth promoting agents such as hormones and antimicrobial agents in food producing animals is critically assessed in the European Union (EU) and controlled by regulations from the European Commission.

3.5 Further application of biodegradable polymers in animal health

The various classes of biodegradable polymers, which differ more or less in their physicochemical properties and degradation behaviour offer the possibility to formulate a range of drugs into a biodegradable delivery system. Indeed only few biodegradable delivery systems, e.g. antiinfectiva [76, 77, 78, 79], vitamin nutritionals [80], antiemetica [81] and cytostatica [82], are described in literature for animals.

Poly(D,L-lactide) microspheres loaded with either ofloxacin or clarithromycin, both macrolides, are examples for the potential of biodegradable polymers to release antibiotic drugs in an advanced manner to animals [76, 77]. Recently, a novel biodegradable injectable gel formulation for the prolonged release of oxytetracycline (OTC) was investigated in sheep [78]. The gel was obtained by adding a great amount of plasticizers to a mixture of different molecular weight PLGA's in which OTC (20 % w/w) was dispersed. The plasma concentration of OTC at or above the minimum inhibitory concentration (MIC) was observed for a period of 6 days. However, only 69 % of OTC loading was released after 15 days and further formulation development will be necessary to achieve complete release and to decrease reaction on injection site.

Currently the Atrigel technology™ was successfully used to develop a dental gel for the treatment of periodontal disease in dogs. The antibioticum doxycycline, a tetracycline derivate, is released from the DL-PLA implant which is formed in situ for at least 7 days (HESKA PERIOceutic Gel™) [83].

Another example for biodegradable antibiotics are PLGA microspheres containing cephradin, a ß-lactam antibiotic which was developed for cattle. Preliminary investigations using dogs showed that therapeutic plasma levels of cephradin were obtained for up to 48 h, although cephradin has a short half-life time of 71 min [79].

Poly(lactide-co-glycolide) was also used for the preparation of a controlled release formulation of a vitamin. Microparticles loaded with Vitamin B12 can be used to improve energy and protein metabolism in animals. A formulation has achieved commercial status and is launched in New Zealand (SmartShot™) [80]. The formulation releases continuously the vitamin for a period over more than 20 days.

Other interesting polymers for veterinary application are injectable semi-solid poly(ortho ester). A paper has recently reviewed their potential in human as well as

animal health [22] and one possible application for companion animals is the treatment of gastrointestinal disorder (GD) in dogs.

Metoclopramide is a useful agent in treating and preventing various types of vomiting, which is one characteristic of GD. Due to the short biological half-life it is usually administered up to four times daily orally in order to maintain therapeutic concentration over the hole day [81]. To prevent fluctuation of plasma level, which produces adverse reactions especially in long-term therapy as well as to improve the compliance, a retard formulation for 3-5 days would be beneficial. This was achieved using a viscous POE to which the drug was added by simply mixing. Preliminary pharmacokinetic results in dogs showed sustained plasma concentration for up to 30 hours. Further development is necessary to prolong the period of drug release.

4. Conclusion and perspectives of biodegradable polymers for veterinary application

Biodegradable polymers have proven their potential use for the development of new, advanced and efficient drug delivery systems. Those are capable of delivering a broad range of bioactive materials in a broad range of veterinary applications.

Suitable therapeutic agents for such biodegradable drug delivery systems are generally those that need to be administered over a long period of time, which are highly active or have a short biological half life such as peptides and proteins.

In the last two decades technological advances have made the production of biodegradable delivery systems more practical and economical. However, until now only few biodegradable delivery systems have entered the market on both human and veterinary side.

The reasons are obvious: At first many drugs such as peptides and proteins are sensitive to heat, shear forces or organic solvents. But those are required for most of the manufacturing processes of classical biodegradable delivery systems such as microspheres or implants. Thus solvent free and sparing methods are of significant interest to avoid stability problems during manufacturing. Furthermore polymers which allow the incorporation of sensitive and/ or instable drugs by simple mixing, without using heat or solvents such as viscous poly(ortho esters) are promising.

Secondly, several factors such as moisture, acidification or interactions between polymer and drug leads to stability problems during storage and release. Last but not

least the often desired zero-order release profile cannot be achieved due to the combination of diffusion and erosion processes. In consequence, the drug release rate varies over the time, especially in the case of long-term applications. Thus, a prediction of the in vivo release based on in vitro data is very difficult and a matter of concern due to the time and cost intensive experiments necessary to development suitable in vitro test systems.

The most important step to overcome this problem is to fully understand the degradation mechanism of applied polymer in order to allow adjusting of release profile. Although systematic degradation studies have been performed especially with aliphatic polyesters the degradation mechanism of these polymers is still not completely understood and demands further investigations.

Nevertheless, in the future many new therapeutic agents will require parenteral application and might benefit from the advantages of biodegradable polymers.

Currently promising biodegradable applications are under investigations for veterinary applications such as guided tissue regeneration, ocular diseases, single-shot vaccination, osteoarthritis or fertility control.

Aims of this Thesis

The research described in this thesis was aimed to investigate a series of low molecular weight poly(D,L-lactides) in order to obtain information about their role in the degradation process of aliphatic polyester which is a controversial subject in literature. Since the solubility of these oligomers is discussed as critical factor in the current theory of bulk erosion and mechanistic degradation studies depending on this issue have not been reported yet it was one aim of this thesis to address this task.

Another aim of this thesis was to investigate the degradation and release characteristics of a branched tartaric acid based polyester, poly(2,3-(1,4-diethyl-co-2,3-isopropyliden)tartrate) (PTA) with respect to its potential use for veterinary drug delivery systems.

A third aim of the thesis was to investigate the possibility to develop different levels of in vitro-in vivo correlation (IVIVC) by using model-dependent and model-independent methods. Due to the fact that drug release from biodegradable delivery

systems occurs by different release mechanisms such as diffusion, dissolution and erosion, IVIVC is still a major problem and a great challenge.

Organization of this thesis

In order to investigate the degradation mechanism and degradation kinetics of low molecular weight poly(D,L-lactides) as function of chain length in **Chapter 2** the synthesis and characterization of a homologous series of low molecular weight poly(D,L-lactides) is described. According to Shih, base-catalyzed hydrolysis should proceed by random scission mechanism, whereas in acid catalyzed hydrolysis chain end scission should be predominant. Since degradation causes an increase in the number of carboxylic acid groups which are thought to auto-catalyze ester hydrolysis, degradation rate should be faster at low pH values.

Chapter 3 reports the incorporation of oligomers into PLGA films in various concentrations by a solvent casting method. The aim of this chapter is to verify the autocatalytic effect of oligomers on the degradation of polymers as reported in literature. Furthermore, the interest is focused on morphological changes during degradation, which could be caused by oligomers.

In **Chapter 4** a less known polyester based on tartaric acid, PTA is characterized in order to investigate the degradation mechanism, which has not reported yet. The polymer contains in contrast to PLGA or PLA additional ester as well as ketal groups in the polymer side chain. It is expected that due to this chemical structure the hydrophobicity of the polymer is increased and thus degradation should be delayed compared to PLGA. In a set of experiments the degradation behavior of PTA implants is monitored regarding to the bulk erosion concept and the morphology of the degrading implants.

In **Chapter 5** the interest is focused on the evaluation of drug release from PTA implants with respect to the potential use of this polymer for veterinary applications. The influence of PTA degradation and erosion is investigated with respect to drug loading, implant size and incorporation of excipients. According to Bengs a small initial drug release is expected which is followed by phase of rather constant drug release.

Chapter 6 reports the preliminary results of the development of a biodegradable implant for veterinary use. The aim of this chapter is to assess the in vitro release

mechanism of buserelin implants which differ in drug loading, coating and copolymer ratio and finally to determine the pharmacokinetic parameters of three selected formulations in dogs. By using different methods such as statistical moment analysis and deconvolution an attempt will be made to develop different levels of correlation.
In the last chapter, the **Conclusion**, the results of this thesis are summarized and some suggestions for future research are presented.

References

[1] Rathbone MJ, Witchey-Lakshmanan L, Ciftci K. Veterinary Application. in: Mathiowitz E. (Ed), Encyclopedia of Controlled Drug Delivery. Wiley, New York, (1999), pp. 1007-1037.

[2] Rathbone MJ, Gurny R. Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam (2000).

[3] Ahmed I, Kasraian K. Pharmaceutical challenges in veterinary product development. Adv. Drug Deliv. Rev. 54 (2002) 871-882.

[4] Witchey-Lakshmanan L, Li Y. Controlled drug delivery in the companion animal. in: Rathbone M, Gurny R (eds), Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam, (2000), pp. 249-267.

[5] Rothen-Weinhold A, Gurny R, Dahn M. Formulation and technology aspects of conrolled drug delivery in animals. Pharm. Sci. Technol. Today 3 (2000) 222-231.

[6] Rathbone MJ, Martinez MN. Modified release drug delivery in veterinary medicine. Drug Discov. Today 7 (2002) 823-829.

[7] Matschke C, Isele U, van Hoogevest P, Fahr A. Sustained-release injectables formed in situ and their potential use for veterinary products. J. Control. Release 85 (2002) 1-15.

[8] Miller J. Controlled release products for control of ectoparasites of livestock. in: Rathbone M, Gurny R (Eds), Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam (2000), pp. 229-248.

[9] Walduck AK, Opdebeeck JP, Benson HE, Prankerd R. Biodegradable implants for the delivery of veterinary vaccines: design, manufacture and antibody responses in sheep. J. Control. Release 51 (1998) 269-280.

[10] Li S, Vert M. Biodegradable Polymers: Polyesters. in: Mathiowitz E. (Ed), Encyclopedia of Controlled Drug Delivery. Wiley John, New York (1999), pp. 71-93.

[11] Vert M, Feijen J, Albertsson A, Scott G, Chiellini E. Biodegradable polymers and plastics. Royal Society of Chemistry, Cambridge (1992).

[12] Goepferich A, Tessmar J. Polyanhydride degradation and erosion. Adv. Drug Deliv. Rev. 54 (2002) 911-931.

[13] Li S,Vert M, Scott G, Gilead D (Eds), Degradable Polymers-Principles and Applications. Chapman and Hall, London, (1995), pp. 43-87.

[14] Pitt CG. Non-microbial degradation of polyesters: mechanism and modifications. in: Vert M, Feijen J, Albertsson A, Scott G, Chiellini E. (Eds), Biodegradable polymers and plastics. Royal Society of Chemistry, Cambridge, (1992), pp. 7-19.

[15] Heller J. Fundamentals of Polymer Science. in: Robinson JR, Lee VHL (Eds), Controlled Drug Delivery Fundamentals and Applications. Marcel Dekker, New York (1987), pp. 139-212.

[16] Krone V, Magerstädt M, Schrinner E, Walch A. DE-OS 3937283, Deutschland, 1991.

[17] Ahlers M, Krone V, Walch A. Microparticles from biodegradable polymers. Adv. Mater. 4 (1992) 230-234.

[18] Bengs H, Bayer U, Krone V, Lill N, Sandow J, Walch A. Polytartrate-a new biodegradable polymer. Proc. Int. Symp. ControI. Release Bioact. Mater. 23 (1996) 114-115.

[19] Heller J, Barr J, Ng SY, Schwach-Abdellaoui K, Gurny R. Poly(ortho esters) synthesis, characterization, properties and uses. Adv. Drug Deliv. Rev. 54 (2002) 1015-1039.

[20] Mao H-Q, Kadiyala I, Ieong KW, Ihao Z, Dang W. Biodegradable polymers: Poly(phosphoester). in: Mathiowitz E (Ed), Encyclopedia of controlled release delivery. (1999), pp. 45-61.

[21] Heller J, Barr J, Ng SY, Shen H-R, Schwach-Abdellaoui K, Emmahl S, Rothen-Weinhold A, Gurny R. Poly(ortho esters) - their development and some recent applications. Eur. J. Pharm. Biopharm 50 (2000) 121-128.

[22] Einmahl S, Capancioni S, Schwach-Abdellaoui K, Moeller M, Behar-Cohen F, Gurny R. Therapeutic applications of viscous and injectable poly(ortho esters). Adv. Drug Deliv. Rev. 53 (2001) 45-73.

[23] Goepferich A. Biodegradable polymers: Polyanhydride. in: Mathiowitz E. (ed), Encyclopedia of Controlled Release Delivery, (1999), pp. 61-71.

[24] von Burkersroda F, Schedl L, Göpferich A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 23 (2002) 4221-4231.

[25] Bunt CR, Woodward VG, Rathbone MJ, Burggraaf C, Ogle CR, Burke CR, Pickering K. A poly (epsilon-caprolactone) bovine intravaginal insert for the delivery of progesterone. Proc. Int Symp. Control. Rel. Bioact. Mater., Boston, 1999, 70-71

[26] Bunt CR, Rathbone MJ, Burggraaf C, Ogle CR, Burke CR. Elevation of plasma progesterone levels in cattle using a poly(epsilon-caprolactone) and cyclodextrin intravaginal insert containing progesterone. Proc. Int. Symp. Control. Rel. Bioact. Mater., Boston, 1999, 1172-1173

[27] Ogle CR, Rathbone MJ, Smith JF, Bunt C, Burggraaf S, Pickering K. Development of an injection moldable, biodegradable intravaginal insert technology. Proc. Int. Symp. Control. Rel. Bioact. Mater., Boston, 1999, 66-67.

[28] Rathbone MJ, Bunt CR, Ogle CR, Burggraaf S, Macmillan KL, Pickering KL. Development of an injection molded poly(epsilon-caprolactone) intravaginal insert for the delivery of progesterone to cattle. J. Control. Release 85 (2002) 61-71.

[29] Johnson CA, Thompson DL. Biodegradable delivery systems for estradiol: comparison between pol(D,L-lactide) microspheres and the SABER delivery system. Proc. Int Symp. Control. Rel. Bioact. Mater., 1999, 147-148.

[30] Burns PJ, Tice TR, Mason DW, Love D, Foss R, Sarver F, Woods J, Sissener T, Heitland A, Wilhelm K, Farlin M, Squires E. Control of estrus and ovulation in mares using progesterone and estradiol biodegradable microspheres in a multicenter clinical trial. Proc. Int Symp. Control. Rel. Bioact. Mater., 1994, 86-88.

[31] Cushman RA, Davis P, Boonyaprakob U, Britt JH, Hedgpeth U. Pharmacodynamic evaluation of biodegradable estradiol-17ß-microspheres and PGF2a for the control of estrus and ovulation in gilts. Proc. Int. Symp. Control. Rel. Bioact. Mater., 1998, 251-252.

[32] Whisnant CS. Effectiveness of slow release steroids on maintenance of serum progesterone concentrations and induction of puperty in heifers. J. Anim. Sci . 77 (Suppl.1) (1999) # 450.

[33] Ogawa Y, Okada H, Heya T, Shimamoto T. Controlled release of LHRH agonist, leuprolide acetate, from microcapsules: serum drug level profiles and pharmacological effects in animals. J. Pharm. Pharmacol. 41 (1989) 439-444.

[34] Riesenbeck A, Klein R, Hoffmann B. Downregulation, eine neue, reversible Möglichkeit zur Ausschaltung der Hodenfunktion beim Rüden. Der Praktische Tierarzt 83 (2002) 512-520.

[35] Sandow J, Jerabek-Sandow G, Krauss B, Schmidt-Gollwitzer M. Pharmacokinetics and metabolism of LHRH agonists, clinical aspects. in: Labrie F, Belanger A, Dupont A. (Eds), LHRH and its analogues. Elsevier Science Publishers B.V., Amsterdam (1984), pp. 123-137.

[36] Ravivarapu HB, Moyer KL, Dunn RL. Sustained Activity and Release of Leuprolide Acetate from an In Situ Forming Polymeric Implant. AAPS PharmSciTech 1 (2000)

[37] Ravivarapu HB, Moyer KL, Dunn RL. Parameters affecting the efficacy of a sustained release polymeric implant of leuprolide. Int. J. Pharm. 194 (2000) 181-191.

[38] Mylonas CC, Tabata Y, Langer R, Zohar Y. Preparation and evaluation of polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH), for inducing ovulation and spermiation in fish. J. Control. Release 35 (1995) 23-34.

[39] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21 (2000) 2475-2490.

[40] www.atrixlabs.com

[41] Capancioni S, Schwach-Abdellaoui K, Zanello P, Guyonnet J, Kaltsatos V, Gurny R. Semi-solid autocatalyzed poly(ortho ester) as a new veterinary drug delivery system for estrus synchronization in ewes. Proc. 4th World Meeting ADRITELV/APGI/APV, Florenz, 2001, 1539-1540

[42] Taylor MA. Recent Developments in Ectoparasiticides. Vet. J. 161 (2001) 253-268.

[43] Lifschitz A, Virkel G, Sallovitz J, Sutra JF, Galtier P, Alvinerie M, Lanusse C. Comparative distribution of ivermectin and doramectin to parasite location tissues in cattle. Vet. Parasitol. 87 (2000) 327-338.

[44] Miller JA, Oehler DD, Pound J. Delivery of ivermectin by injectable micropheres. J. Econ. Entomol. 91 (1998) 655-659.

[45] Miller JA, Davey RB, Oehler DD. Control of cattle fever ticks using injectable microspheres containing ivermectin. Proc. Int. Symp. Control. Rel. Bioact. Mater., Boston, 1999, 1166-1167.

[46] Miller JA, Davey RB, Oehler DD, Pound J, George J, Ahrens E. Control of Boophilus annulatus (Say) (Acari; Ixodidae) on cattle using injectable microspheres containing ivermectin. J. Econ. Entomol. 92 (1999) 1142-1146.

[47] www.proheart.com

[48] Genchi C, Rossi L, Cardini G, Kramer L, Venco L, Casiraghi M, Genchi M, Agostini A. Full season efficacy of moxidectin microsphere sustained release formulation for the prevention of heartworm (Dirofilaria immitis) infection in dogs. Vet. Parasitol. 110 (2002) 85-91.

[49] Rock D, Heaney K, Peterson D, Barton W, Levy S, Smith L, Terhune T. Field evaluation of a moxidectine sustained release injectable for the prevention of heartworm disease in dogs. Proc. Am. Assos. Vet. Parasitol., 2000, 56.

[50] Butler J, Vaughan J, Rulli R. Clinical observations following the administration of moxidectine canine sustained release (SR) injectable in heartworm positive dogs. Proc. Am. Assos. Vet. Parasitol., 2000, 57.

[51] Jaffe H, Miller J, Giang P, Hayes D. Implantable systems for delivery of insect growth regulators to livestock. Proc. Int. Symp. Control. Rel. Bioact. Mater., 1980, 237-250.

[52] Jaffe H, Giang P, Hayes D, Miller J,Stroud B. Implantable systems for delivery of insect growth regulators to livestock. in: Lewis DH. (Ed), Controlled release of pesticides and pharmaceuticals. Plenum, New York, (1981), pp. 303-310.

[53] Jaffe H, Hayes D, Dees W, Beveridge M, Thompson M. Controlled-release reservoir systems for the delivery of insect steroid analogues against ticks. J. Med. Entomol. 23 (1986) 685-691.

[54] Shih C, Fix J,Seward R. In vivo and in vitro release of ivermectin from poly(ortho ester) matrices. I.Crosslinked matrix prepared from ketene acetal end-capped prepolymer. J. Control. Release 25 (1993) 155-162.

[55] Carino G. Vaccine Delivery. in: Mathiowitz E. (Ed), Encyclopedia of Controlled Drug Delivery. Wiley, New York (1999), pp. 993-1006.

[56] O'Hagan D, Singh M, Gupta R. Poly(lactide-co-glycolide) microparticles for the development of single-dose controlled-release vaccines. Adv. Drug Deliv. Rev. 32 (1998) 225-246.

[57] O'Hagan D. Microparticles and polymers for the mucosal delivery of vaccines. Adv. Drug Deliv. Rev. 34 (1998) 305-320.

[58] Vajdy M, O'Hagan D. Microparticles for intranasal immunization. Adv. Drug Deliv. Rev. 51 (2001) 127-141.

[59] Eldridge J, Staas J, Meulbroek J, McGhee T, Tice R, Gilley R. Biodegradable microspheres as a vaccine delivery system. Mol. Immunol. 28 (1991) 287-294.

[60] Bowersock T, Martin S. Controlled release vaccines in veterinary medicine. in: Rathbone M, Gurny R. (Eds), Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam (2000), pp. 269-309.

[61] Greenway T, Eldridge J, Ludwig G, Staas J, Smith J, Gilley R, Michalek S. Enhancement of protective immune response to Venezuelan equine encephalities (VEE) virus with microencapsulated vaccine. Vaccine 13 (1995) 1411-1420.

[62] Barr I, Kleining M, Thiel W. A single-dose vaccination system of inactivated veterinary vaccine. International Congress on the Regulation of Leukocyte Production and Immune Function, Sydney, 1993.

[63] Hazrati A, Lewis D, Atkins T, Stohrer R, McPhillips C, Little J. Salmonella enteritidis vaccine utilizing biodegradable microspheres. Proc. Int. Symp. Control. Release Bioact. Mat., 1993, 101-102.

[64] Estunigsih S, Smooker P, Wiedosari E, Widjajanti S, Vaiano S, Partoutomo S, Spithill T. Evaluation of antigens of Fascioloa gigantica as vaccine against tropical fasciolosis in cattle. Int. J. Parasitol. 27 (1997) 1419-1428.

[65] www.teagasc.ie/publications//livefluke.html

[66] Ertl H, Varga I, Xiang Z, Kaiser K, Stephens L, Otvos L. Poly(D,L-lactide-co-glycolide) microspheres as carrier for peptide vaccines. Vaccine 14 (1996) 879-885.

[67] Allaoui-Attarki K, Pecquet S, Fattal E, Trolle S, Chachaty E, Couvreur P, Andremont A. Protective immunity against Salmonella thyphimurium elicited in mice by oral vaccination with phosphoylcholine encapsulated in poly(D,L-lactide-co-glycolide) microspheres. Infect. Immun. 65 (1997) 853-857.

[68] Allonso M, Cohen S, Park T, Gupta R, Siber G, Langer R. Determinants of release rate of tetanus from polyester microspheres. Pharm. Res. 10 (1993) 945-953.

[69] Men Y, Thomasin C, Merkle H, Gander B, Corrandin A. A single adminitration of tetanus toxoid biodegradable microspheres elicit T cells and antibody responses similar or superior to those obtained with aluminium hydroxide. Vaccine 13 (1995) 68-89.

[70] Preston RL. Hormone containing growth promoting implants in farmed livestock. Adv. Drug Deliv. Rev. 38 (1999) 123-138.

[71] Foster TP. Somatropin delivery to farmed animals. Adv. Drug Deliv. Rev. 38 (1999) 151-165.

[72] Foster T, Moseley W, Caputo J, Alaniz G, Leatherman M, Yu X, Claflin W, Reeves D, Cleary M, Zantello R. Sustained elevated serum somatotropin concentrations in Holstein steers following subcutaneous delivery of a growth hormone releasing factor analog dispersed in water, oil or microspheres. J. Control. Release 47 (1997) 91-99.

[73] Wyse JW, Takahashi Y, DeLuca PP. Instability of porcine somatotropin in polyglycolic acid microspheres. Proc. Int. Symp. Control. Rel. Bioact. Mater., 1989, 334-335.

[74] Thompson WW, Anderson DB, Heiman ML. Biodegradable microspheres as a delivery system for rismorelin porcine, a porcine-growth-hormone-releasing-hormone. J. Control. Release 43 (1997) 9-22.

[75] Conrad JM, Skinner DS. Controlled sustained delivery of monensin in cattle: monensin R.D.D. J. Control. Release 9 (1989) 133-147.

[76] Bahk JY, Hyun JS, Lee JY, Kim J, Cho YH, Lee JH, Park JS, Kim MO. Concentration of oflocacin in canine tissue and prostate: fluid after intraprostatic injection of biodegradable sustained-releasing microspheres. J. Urol. 163 (2000) 1560-1564.

[77] Gupta PK, Johson H, Allexon C. In vitro and in vivo evaluation of clarithromycin PLA microspheres for intramuscular drug delivery. J. Control. Release 26 (1993) 229-238.

[78] Sun Y, Peng Y, Aksornkoae N, Johnson JR, Boring JG, Scruggs D, Cooper RC, Laizure SC, Shukla AJ. Controlled release of oxytetracycline in sheep. J. Control. Release 85 (2002) 125-134.

[79] Ustariz-Peyret C, Vert M. Labile conjugation of a hydrophilic drug to PLA oligomers to modify a drug delivery system: cephradin in a PLAGA matrix. J. Microencaps. 17 (2000) 615-624.

[80] www.stockguard.co.nz

[81] Schwach-Abdellaoui K, Moreau M, Schneider M, Boisram B, Gurny R. Controlled delivery of metoclopramide using an injectable semi-solid poly(ortho ester) for veterinary application. Int. J. Pharm. 248 (2002) 31-37.

[82] Verrijk R, Smolders IJ, Bosnie N, Begg AC. Reduction of systemic exposure and toxicity of cisplatin by encapsulation on poly-lactide-co-glycolide. Cancer Research 52 (1992) 6653-6656.

Chapter 2

Characterization of a homologous series of D,L-lactic acid oligomers:

A mechanistic study on the degradation kinetics in vitro

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Abstract

A series of low molecular weight polymers of D,L-lactic acid has been synthesized. The oligomers were characterized with respect to molecular weight, glass transition temperature and solubility. The number average molecular weight of the oligomers ranged from 290 to 1320 Da. Oligomers with an $\overline{M}_n$ < 800 Da were soluble in buffer at pH 7.4 but insoluble in water and acidic medium.

Kinetic studies were performed at pH 1.5, 4.5 and 7.4 using an accelerated in vitro monomer release test. The average hydrolytic rate was dependent on molecular weight of oligomer, temperature and pH of the media, with the lowest rate found around pH 4.5. The activation energy was dependent on molecular weight and ranged from 47 to 67 kJ mol^{-1}.

Chain-end cleavage ("unzipping") was identified as mechanism of hydrolysis in acidic media whereas random ester and/or backbiting at the chain ends were the possible mechanism of hydrolysis in basic media.

1. Introduction

Poly(lactic acid) (PLA) and its copolymers with poly(glycolic acid) (PGA) are widely used for pharmaceutical and biomedical applications because they are biodegradable and biocompatible [1]. An important attribute of these polymers is the possibility to modulate the degradation rate of a delivery system by changing e.g. chemical composition (homo- or copolymers of lactic and glycolic acid) or the physical properties (molecular weight, glass-transition temperature) and consequently to control the drug release [2]. Therefore, the degradation mechanism of polyesters has been a subject in numerous investigations [3-7]. The results of these studies are somewhat controversial and until now the role of low molecular weight degradation products (oligomers) in the degradation process is not fully understood. Generally the hydrolytic degradation of polyesters in aqueous media proceeds through random ester bond cleavage in the bulk of the device [4, 8, 9]. This process is affected by four principal parameters, namely rate constant, amount of absorbed water, diffusion coefficient of chain fragments within the polymer matrix and solubility of degradation products in the surrounding medium.

The most common explanation for this heterogeneous degradation process is as follows [10-12]: degradation starts with the absorption of water, followed by the hydrolytic cleavage of ester bonds, which generates chain fragments with acidic end groups. This process is characterized by a decrease in molecular weight, an increase in the polydispersity P (= M_w/ M_n) and a lack of polymer mass loss. In the initial degradation phase the cleavage of ester bonds occurs preferentially at the surface of the device due to the gradient of absorbed water. After a short period of time this gradient disappears, because water diffusion is relatively rapid in comparison to polymer-chain degradation. The discovery of a faster degradation inside larger devices greatly changed the understanding of polymer degradation. The heterogeneous degradation was assigned to ”reaction/diffusion phenomena” which were identified to govern polymer degradation [12, 13]. These phenomena involve water soluble, low molecular weight degradation products, which are formed at the surface as well as in the inner part of the device. In contrast to small size devices where soluble oligomers can escape before they are totally degraded, in large size devices only soluble oligomers located close to the surface are extracted whereas those located inside the device remain entrapped due to the relatively small diffusion

coefficients of oligomers. In consequence the concentration of carboxylic end groups is higher in the center than at the surface and thus increasing the degradation rate. Therefore, it has been suggested that the degradation of large devices leads to a surface-center differentiation due to the phenomena described above, also designated as “autocatalysis”. It is also known that ions from the medium decrease the relative acidity of the surface and form an acidity gradient from surface to center, which contributes to such differentiation [12, 14].

It was also postulated that oligomers produced during the hydrolysis create an osmotic pressure between the interior of the device and the surrounding medium, which can be explained as well by the ”reaction/ diffusion phenomenon” [15]. This osmotic pressure draws water into the matrix and the outer layer acts as “semipermeable membrane”. All effects lead to a faster degradation in the center than at the surface. It is assumed that if internal degradation products become small enough to be soluble (critical molecular weight of oligomer) and the surface becomes permeable they can escape, in parallel mass loss is detected. With increasing polymer chain length more bonds have to be cleaved in order to generate water-soluble oligomers, therefore time until onset of mass loss increases.

Whether bulk or surface erosion occurs depends on the formation velocity of diffusing oligomers [16]. Recently it has been reported that depending on device geometry degradation can shift from bulk to surface erosion [17].

In the literature only scant information is available on the critical molecular weight for water solubility of oligomers and their degradation behaviour [18-20]. The numbers range from 1500 Da up to 5000 Da [21-23]. Furthermore, only few reports dealing with the effect of oligomers with various molecular weights on the degradation profile of polymer devices have been published [20, 24, 25].

The objective of this paper was to synthesized a series of oligo-D,L-lactides with narrow molecular weight distribution and to characterize them with respect to their physico-chemical properties as function of average chain length. To obtain information about the degradation kinetics of oligomers the degradation was monitored using either an accelerated monomer release test based on high performance liquid chromatography (HPLC) or nuclear magnetic resonance spectroscopy (NMR). The paper gives new information about oligomer properties which should be helpful to understand degradation of polymers.

2. Materials and methods

2.1 Materials

90 % D,L-lactic acid (LA) in aqueous solution was obtained from Sigma (Germany). Tetrahydrofuran (THF) in spectroscopic grade, dichloromethane (DCM) and ethanol were purchased from SDS (Germany). Deuterated acetone (aceton-d_6), deuterium oxide (D_2O), deuterochloric acid (DCl, 20 % in D_2O), acetonitrile-d_3 (ACN-d_3), 4-N,N-dimethyl-
aminopyridine (DMP), phenolphthalein, trifluoroacetic acid (TFA), petroleum ether and 0.1 N sodium methylat solution ($NaOCH_3$) were obtained from Merck (Germany). All materials used were of analytical purity.

2.2 Synthesis of D,L-lactic acid oligomers

Polydisperse lactic acid oligomers with varying average molecular weights were synthesized according to [26]. 100 ml of 90 % D,L-lactic acid aqueous solution was allowed to concentrate by gentle distillation of water. The reaction started at normal pressure and was then changed to reduced pressure after removal of water. The temperature was slowly increased to reach 140 °C after 3 days. At various time points of polymerization a sample was taken and analyzed for number average molecular weight ($\overline{M}_n$). The synthesis times ranged between 4 hours and 8 days. The yield of oligomer production ranged between 56 and 83 %. All samples were stored in a desiccator at room temperature.

2.3 Characterization of oligomers

2.3.1 End group analysis

The quantity of carboxylic group in each oligomer was determined by titration of the oligomer solution with 0.1 N $NaOCH_3$. Each sample was dissolved in DCM and diluted with ethanol (2 % w/ v). Phenolphthalein was added and the solution was titrated with 0.1 N $NaOCH_3$. The volume of alkali solution consumed for titration was used to calculate the number average molecular weight of the oligomer by using Eq. (1) [27]:

$$\overline{M}_n = \frac{W}{C \times (V - V_0)} \quad (1)$$

where W is the weight of the oligomer titrated. C is the concentration of the $NaOCH_3$ solution. V and V_0 are the volume of the $NaOCH_3$ solution used for titration of oligomer solution and blank, respectively. Results are the mean of 5 determinations.

2.3.2 Size exclusion chromatography

The molecular weights and molecular weight distributions of polydisperse D,L-lactic acid oligomers were determined by SEC using styragel columns (PSS guard column SDV, PSS SDV 100, 5 µm, 300 x 7.5 mm, Polymer Standard Service, Mainz, Germany)[28]. Degassed THF containing 0.1 % (v/ v) trifluoroacetic acid (TFA) was used as the mobile phase at a flow rate of 1 ml/ min. An oligomer aliquot was dissolved in THF and filtrated before injection. The average molecular weight was determined relative to polystyrene standards (Polymer Standard Service, Mainz, Germany) using refractive index detection at 30 °C.

2.3.3 NMR spectroscopy

For ^{1}H-NMR spectroscopy a polar solvent was used for the dissolution of oligomers as recommend by Espartero et. al [7]. Samples were dissolved in acetone-d_6 (1 mg/ ml) and the spectra were recorded on a Jeol spectrometer operating at 500 MHz at room temperature (16 scans).

Chemical shifts (δ) were expressed in ppm with the acetone-d_6 signal at 2.06 ppm as a reference. The average degree of polymerization ($\overline{DP}_n$), $\overline{M}_n$ and the monomer content were determined from the ratio of integrals of different α-methyl proton signals (δ 1.2-1.5 ppm) described in [7].

2.3.4 Differential scanning calorimetry

Measurements of glass transition temperatures (T_g) were performed using a differential scanning calorimeter (DSC 821, Mettler Toledo, Greifensee, CH). Two samples (approx. 7 mg) of each oligomer were heated twice under nitrogen atmosphere. Thermograms covering a range of -60 °C to 200 °C were recorded at heating and cooling rate of 10 K/ min. The second DSC scan run was used for T_g

calculation (onset method). Calibration of the system was performed using gallium and indium standards.

2.4 In vitro degradation

From an analytical viewpoint several techniques such as SEC, HPLC, capillary zone electrophoresis or mass spectrometry can be used for monitoring degradation in vitro [29-32].

In this work degradation was measured by the appearance of lactic acid in the release medium using HPLC. It should be note, that the applied test could be used in combination with e.g. mass spectroscopy for more quantitative determination of degradation profiles.

The monomer release studies were carried out in buffer solutions at pH 1.5, 4.5 and 7.4. 50 mg of each oligomer (n=5) were placed in an iodine flask containing 25 ml of selected medium and incubated at 65 °C. The activation energies were determined by conducting studies at pH 7.4 at 70, 80 and 90 °C.

At defined time intervals 2 ml of medium were withdrawn and replaced by fresh medium. Samples were frozen until the study was completed and analyzed for lactic acid content by HPLC according to the procedure described in [33]. The following modifications were applied. Separation was performed on a C 18 column (250 x 4.6 mm I.D., Machery Nagel, Düren, Germany) fitted with a guard column. The mobile phase, phosphate buffer (pH 2.9) was used with a flow gradient. The UV-VIS detector (Waters 2470, Waters Corporation, Milford, MA) was set at 210 nm. The column temperature was maintained at 35 °C using a column oven (Jet Stream, Waters Corporation, Milford, MA). All samples were stored at 6 °C in the autosampler.

Calibration curves were generated from known concentrations of D,L-lactic acid in buffered media. The detection limit was 5 µg/ml.

2.5 Hydrolysis mechanism

In accordance with previous experiments performed by the group of Shih et al. [34, 35] the hydrolysis mechanism was investigated using either DCL or DMP as catalyst. Each oligomer sample (approx. 20 mg) was dissolved in ACN-d_3 (approx. 1.5 ml) in a volumetric flask (2 ml). Then 0.2 ml of DCl (1.18 M in D_2O) or 0.5 ml of DMP (0.933 M in D_2O) was added, respectively. The flask was finally filled with ACN-d_3.

The concentration of DCl and DPM in the reaction mixture was 0.118 M and 0.223 M, respectively. An aliquot (approx. 0.8 ml) of the solution was immediately transferred to a NMR tube and sealed. Spectra were taken as described above over a 24 h period at room temperature. Chemical shifts were expressed in ppm referencing to the ACN-d_3 signal at 1.96 ppm.

3. Results and discussion

3.1 Characterization

A series of oligo(D,L-lactic acids) were synthesized by direct step-growth polymerization of D,L-lactic acid without any catalyst. The oligomers obtained, designated OLG 1 to 5, appeared clear to slightly yellowish colored and had a liquid, waxy or solid consistence depending on the synthesis time, which varied from 4 hours to 8 days. Yields were found to be between 56 and 83 %.

The oligomers were first characterized by end-group analysis, SEC, DSC and ^{1}H-NMR. The obtained molecular and thermal properties are summarized in Table 1. The number-average molecular weight ranged from 290 to 1320 Da according to an average degree of polymerization from 4 to 18. The DSC experiments showed that all polydisperse oligomers are amorphous waxes with glass transition temperatures between -17.2 °C and 27.2 °C. This dependence of T_g on molecular weight is well known and described e.g. in [36]. Furthermore, it was observed that T_g increased

Table 1. Characterization of polydisperse D,L-lactic acid oligomers

Code	$\overline{DP}_n$ [a]	$\overline{M}_n$ [a]	$\overline{M}_w$ [b]	$\overline{M}_w / \overline{M}_n$ [c]	T_g (°C)[d]	LA (%)[a]	attribute
OLG 1	4	290	280	1.3	-17.2	7.7	liquid
OLG 2	5	380	430	1.4	3.5	7.3	viscous
OLG 3	7	540	630	1.6	11.8	4.5	viscous
OLG 4	11	830	960	1.8	15.6	3.5	solid
OLG 5	18	1320	1560	2.3	27.2	2.7	solid

[a] obtained by H-NMR
[b] obtained by end-group analysis
[c] obtained by SEC
[d] obtained by DSC

strongly at the beginning of polycondensation due to the change in concentration of the low molecular weight fraction and water in the reaction mixture. In the course of the reaction the molecular weight increased whereas the water content and the monomer concentration decreased. ^{1}H-NMR was used to determine residual monomer in the oligomers [7].

The solubility of oligomers which has up to now not been reported was tested in various solvents. All oligomers were found to be soluble in acetone, DCM and THF but insoluble in petroleum ether, demineralized water and 0.01 N HCl. In phosphate buffer at pH 7.4 all oligomers were soluble with the exception of OLG 4 and 5, which appeared swollen after 48 hours. The different solubilities in aqueous media result from the pk_a of lactic acid (pk_a 3.4) [37]. At neutral pH the free carboxylic end group is dissociated and forms a salt, which is soluble depending on the molecular weight of the oligomer. If the concentration of hydrogen ions increases and reaches values below pH 3 the undissociated, more lipophilic free acid function is formed. Ionization of the carboxylic end groups was necessary for hydration and solubility. The ratio of end-group to oligomer chain length increases with decreasing molecular weight, resulting in an increased solubility of oligomer. This ratio reached the highest value in OLG 1 ($\overline{M}_n$ 290 Da) and the smallest in OLG 5 ($\overline{M}_n$ 1320 Da) which explains the obtained differences in solubility.

3.2 Effect of temperature on degradation kinetics of lactic acid oligomers

The degradation experiments with D,L-lactic acid oligomers for determination of activation energy were carried out in aqueous phosphate solution (pH 7.4) at four temperatures (65, 70, 80 and 90 °C).

The degradation of oligomers resulted in fragments with hydroxylic and carboxylic end groups, which finally degraded to the monomer lactic acid. The increase of monomer content in the buffer medium was used as an indicator for the extent of hydrolysis and was monitored by HPLC. The fraction of oligomers, which has still to degrade to the monomer was expressed as remaining monomer. The percentage of remaining monomer was plotted against the time on a semi-logarithmic scale. From the slope of the plot the hydrolytic cleavage rate constant k_{obs} was calculated. It was found that with increasing $\overline{DP}_n$ the reaction rate constant decreased in parallel with a visually observed decrease in solubility of oligomer in the medium. Furthermore, for

each oligomer k_{obs} increased with increasing incubation temperature. This temperature dependency of the reaction rate is described by the Arrhenius equation (Eq.(2)) as follows:

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \qquad (2)$$

where k_{obs} is the observed reaction rate constant, A is the frequency factor and E_a is the activation energy, R is the universal gas constant (8.3143 Joule K^{-1} mol^{-1}) and T is the absolute temperature. Eq. (2) indicates that a graph of ln k_{obs} versus 1/ T is linear with a slope of $-E_a/ R$ and an intercept of ln A (Fig. 1). The calculated E_a of hydrolysis for four oligomers ranges from 47 to 67 kJ/ mol. The E_a obtained for OLG 5 is similar to results reported by Dunn et al. [38] and Makino et al. [6]. However, E_a values in the literature vary over a broad range because of dissimilarities in molecular weights and polymer composition [39-41].

It was found that oligomers having smaller average molecular weights show smaller E_a values (47.0, 49.1 and 51.5 kJ/ mol corresponding to OLG 1, 2 and 3). An additional explanation could be the higher hydrophilicity of OLG 1, 2 and 3 in comparison to OLG 5, being less capable of absorbing water. Swelling through water

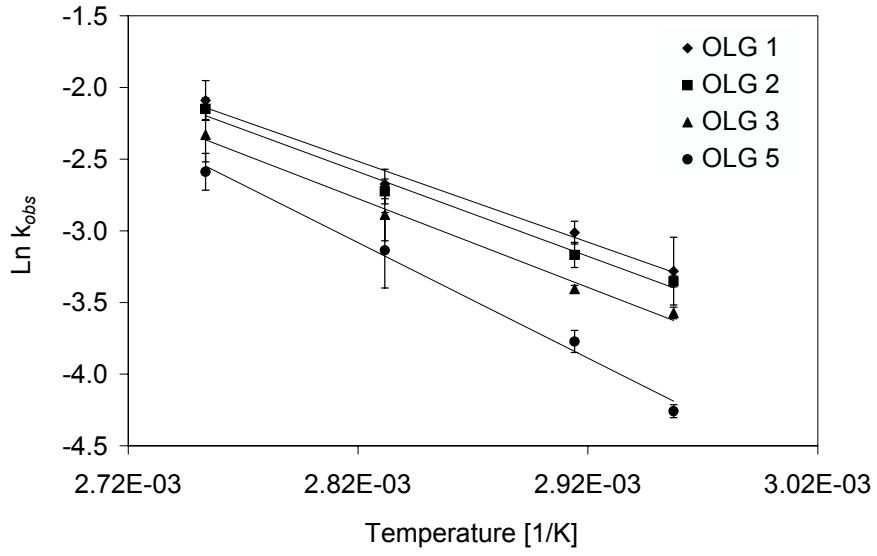


Figure 1. Arrhenius plots for the degradation rates of various D,L-lactic acid oligomers obtained at four incubation temperatures (65, 70, 80 and 90 °C).

absorption leads to an increase of the theoretical free volume and chain flexibility within the matrix thus facilitating hydrolysis. In consequence less energy of activation is necessary to cleave ester bonds.

3.3 Effect of pH on degradation kinetics and mechanism of hydrolysis

The effect of pH on the hydrolysis rate constant of various D,L-lactic acid oligomers was analyzed by monitoring the release of lactic acid in the surrounding medium at 65 °C by HPLC.

The percentage of remaining monomer was plotted against time in a semi-logarithmic scale. From the slope of the line the reaction rate constant (k_{obs}) was obtained. The concentration of monomer in the samples increased according to pseudo first-order kinetics for the studied pH values and is exemplarily shown for OLG 2 (Fig. 2).

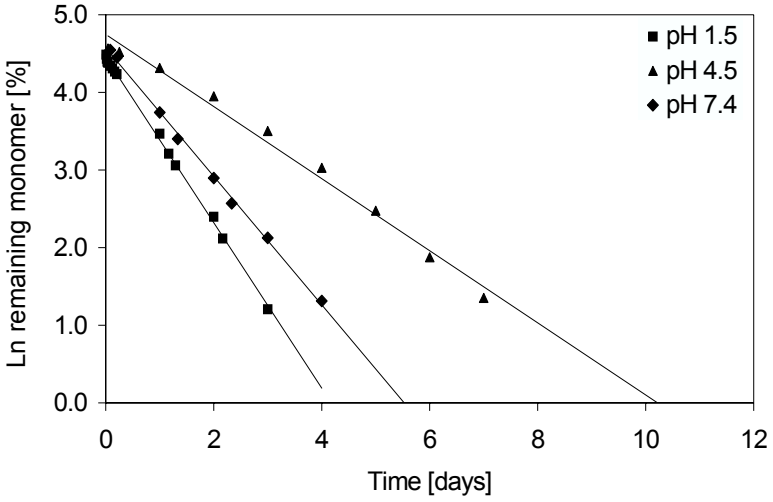


Figure 2. Degradation profile of OLG 2 depending on pH at 65 °C

At all three pH values under investigations, i.e. 1.5, 4.5 and 7.4, monomer release was fastest from the smallest oligomer as expected. For all oligomers the monomer release was slowest at pH 4.5. These results are in accordance with the mechanism of acid/ base catalyzed hydrolysis [6]. The pH profiles of the experimentally determined rate constants for various oligomers are shown in Fig. 3. Around the pk_a value of lactic acid (pk_a 3.4) the reaction rate of oligomers had reached the minimum. This finding is in accordance with results obtained in a more detailed investigation of the pH dependency of k_{obs} performed by DeJong et al. [25] and Maniar et al. [18].

Our results regarding E_a and the log k_{obs} – pH profile provided evidence for the suitability of employing the in vitro monomer release test to investigate the degradation kinetics of oligomers as function of pH, temperature and average chain length. Due to the fact that this test was based exclusively on the detection of released monomer related mainly to the terminal ester bonds it provides limited information on the degradation mechanism.

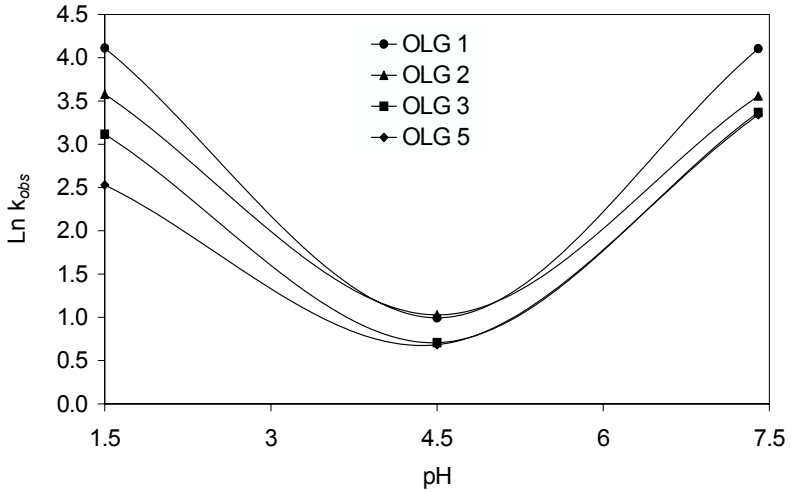
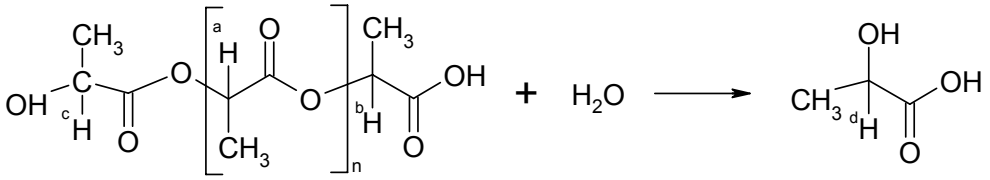


Figure 3. Ln k_{obs}-pH profile of D,L-lactic acid oligomers.

^{1}H-NMR spectroscopy allows direct monitoring of ester bond cleavage and is capable of differentiating exactly between terminal and internal ester bonds.

In Scheme 1 the degradation of poly(D,L-lactic acid) is described. Four different types of methine proton (-CH) signals (δ 4.0-5.2 ppm) were identified (Fig. 4) in accordance with DeJong et al. [32]. Multiplets at 5.2 ppm, 5.07 ppm and 4.2 ppm were assigned to the methine proton signal of internal ester bonds (-CH^a), ester bonds at the carboxylic chain-end (-CH^b) and ester bonds at the hydroxylic chain end (-CH^c), respectively. The methine proton of free lactic acid (-CH^d) appeared as quartet at 4.08 ppm. The abbreviation M is used from now on as synonym for the methine proton signals. From the signal intensities the peak area of each methine proton was calculated as follows.

The fraction of total ester bonds (= fraction of unhydrolyzed ester bonds) at a time point was obtained from the area ratio of $(M_1+M_2)/ (M_1+M_2+M_3+M_4)$. The fraction of



Scheme 1

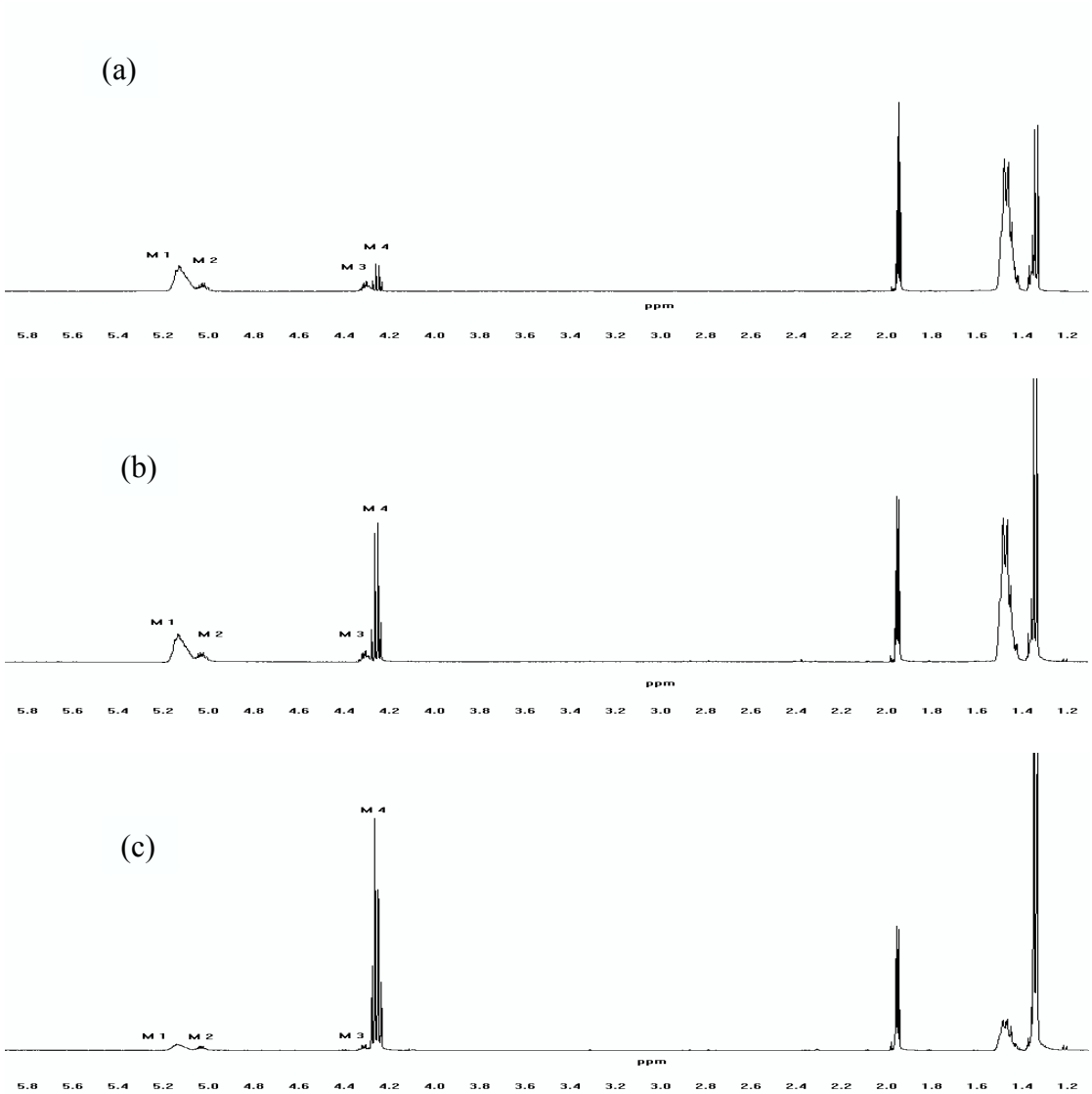


Figure 4. ^{1}H-NMR spectra taken after (a) 0.5 h, (b) 8 h and (c) 24 h of OLG 2 hydrolysis catalyzed by 0.118 M DCl in ACN-d_3.

free lactic acid was calculated from the area ratio of M_4/ (M_1+M_2+M_3+M_4) and the fraction of terminal ester bonds resulted from M_3/ (M_1+M_2+M_3+M_4). During degradation occurred a change in these peak areas. The peak area of internal ester bonds (M_1) decreased while those of terminal ester bonds (M_2 and M_3) and of free lactic acid (M_4) increased. By monitoring these changes it is possible to discriminate between the mechanism of hydrolysis, i.e. random or chain-end cleavage. If the formation of monomer is proportional to loss of ester bonds, chain-end cleavage is the mode of hydrolysis. If, in contrast, ester bond cleavage occurs randomly the formation of monomers is not proportional to the loss of ester bonds. In consequence $\overline{DP_n}$ declines faster than by chain-end hydrolysis.

In Fig. 5 the fraction of the four methine proton signals of various oligomers at the beginning of experiment is shown. It should be emphasized that the initial composition obtained in acidic medium was identical to that found under basic conditions. The areas of M2 and M3 were similar and ranged from 19 % to 5 % depending on the oligomers studied. The fraction of M4 (monomer) was the greatest (7.5 %) in the oligomer sample with the lowest average molecular weight and decreased with increasing average chain-length to 2 %.

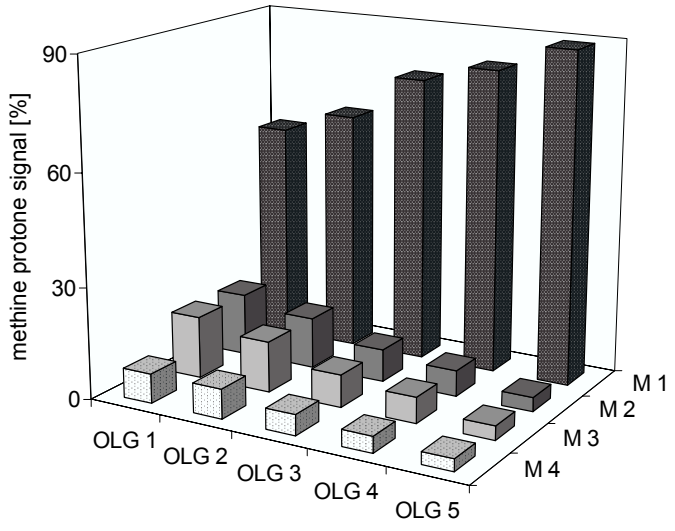


Figure 5. Fraction of methine proton signals from various oligomers in ACN-d_3 obtained at the beginning of experiment.

The fraction of M_1 (internal ester bonds) was the smallest in OLG 1 and increased with raising average molecular weight.

In Fig. 6a the ratio of methine proton signals obtained in acid (0.118 M DCl) after 24 h incubation is shown. The fraction of M_4 increased depending on $\overline{DP}_n$ of oligomers whereas the fraction of M_1 decreased only slightly. It was found that the loss of ester bonds was independent from the total ester bonds (E) and followed a pseudo-zero order kinetics (Fig. 6b) as given by Eq.(3)

$$-d[E]/ dt = k^0/ DP_n^0 \quad (3)$$

where k^0 is the pseudo-zero order rate constant and DP_n^0 is the initial average degree of polymerization.

The plot of total ester bonds and monomer content versus time is exemplarily shown for OLG 4 in Fig. 6c. An excellent linearity ($0.9935 \leq R^2 \leq 0.9951$) over degradation time was obtained. The lines run almost parallel and the slopes are similar. In contrast, a plot of total ester bonds and terminal ester bonds (sum of M_2 and M_3) as function of time does not show any correlation (Fig. 6c, insert). The zero order degradation data obtained under acidic conditions for various oligomers prove that hydrolysis proceeds via *chain-end cleavage* ("unzipping") as described by Shih et al. [34].

Using N,N-dimethylaminopyridine (DMP) as basic catalyst another hydrolysis mechanism was found. The ratio of methine proton signals after 24 h incubation is shown in Fig. 7a. The fraction of M_1 decreased strongly in parallel with an increase of M_2 and M_3 whereas M_4 increased less than under acidic conditions. In contrast to results obtained in acidic media it was found that in alkaline media the loss of ester bonds depends on the total ester bonds and followed a pseudo-first order kinetics (Fig. 7b) which is described in Eq.(4)

$$-d[E]/ dt = k^1 E \quad (4)$$

where k^1 is the pseudo-first order rate constant.

Moreover, it looks that the plot of total ester bonds and terminal ester bonds versus time follow the same profile (Fig. 7c). However, due to the fact that at an infinite degradation time only the monomer is left in the H-NMR spectrum, the amount of terminal ester bonds should display a maximum before they reduce to zero. Therefore

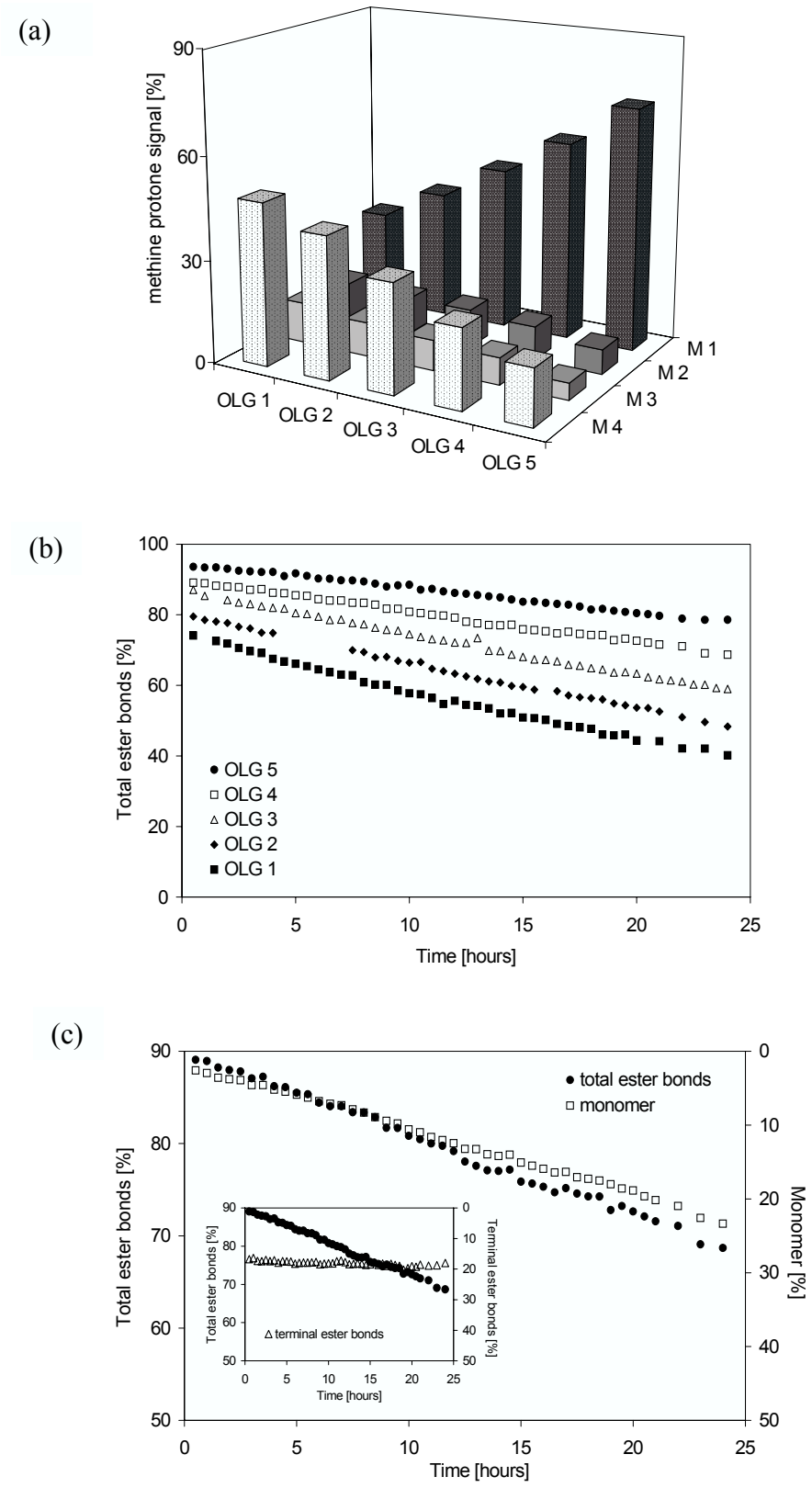


Figure 6. Acid hydrolysis: (a) fraction of methine proton signals from various oligomers after 24 h, (b) plot of total ester bonds versus time, (c) OLG 4: plot of total ester bonds and monomer as well as terminal ester bonds (Fig. insert) versus time.

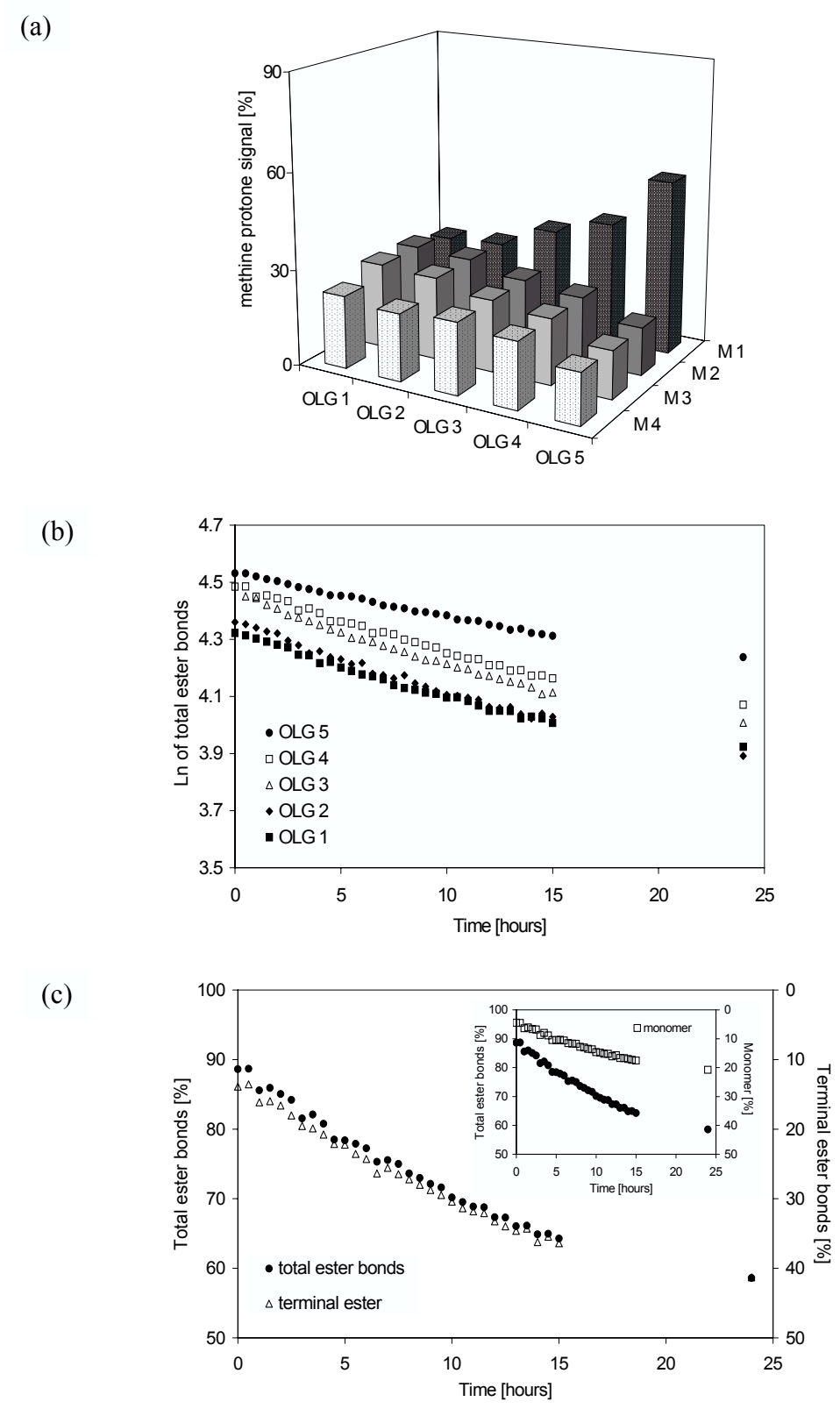


Figure 7. Basic hydrolysis: (a) fraction of methine proton signals from various oligomers after 24 h, (b) plot of ln total ester bonds versus time, (c) OLG 4: plot of total ester bonds and terminal ester bonds as well as monomer (Fig. insert) versus time.

the plot of terminal ester bonds versus time can not be continuously linear or follow 1st order kinetics.

Since it is most likely that under the applied conditions cyclic dimers were formed as intermediate during hydrolysis [25] which show a peak at the same chemical shift as the methine protone signal near the terminal carboxylic acid bonds, the plot in Fig 7 c compares not only the formation rate of terminal ester bonds but also the formation rate of cyclic lactides with the degradation rate of total ester bonds. The lines run parallel with similar slopes which suggest that the hydrolysis of oligomers occurs not by a chain-end cleavage. Moreover, it seems that degradation under basic conditions proceeds by backbiting at the chain ends as described by DeJong et al. [25] and not really randomly as proposed for e.g. poly(lactic acid) [35].

As shown in Fig. 7c, insert the number of total ester bonds decreased faster than the formation rate of monomer which is probably due to random ester cleavage. However it can also be explained by the formation of cyclic lactides by the backbiting mechanism.

As previously mentioned, a random ester cleavage leads to a faster decline of $\overline{DP}_n$ because hydrolysis under alkaline conditions is irreversible. Under acidic conditions hydrolysis is reversible and therefore somewhat slower. Furthermore, chain-end cleavage reduces the polymer chain length step by step about one monomer only. In contrast, random ester cleavage cuts the polymer chain randomly i.e. anywhere in the chain, thus reducing $\overline{DP}_n$ evidently. In consequence, $\overline{DP}_n$ declines faster.

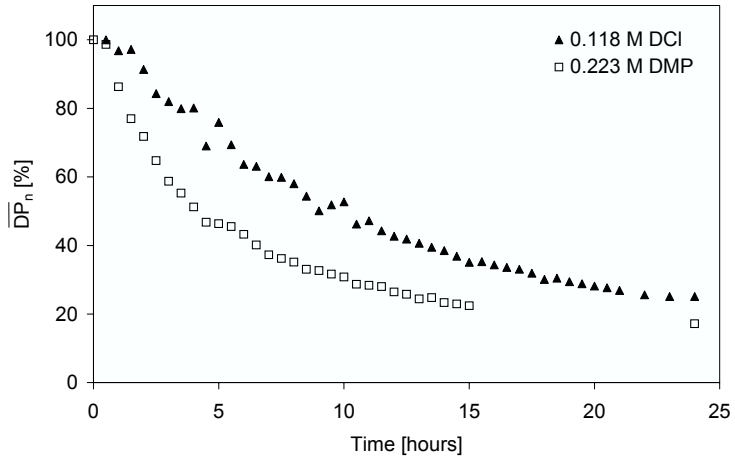


Figure 8. Plot of absolute $\overline{DP}_n$ versus the time as function of catalyst for OLG 5.

However, it should be mentioned that the degradation rate is highly pH-dependent and therefore the difference can be ascribed also to pH and not only to the mechanism.
Fig. 8 shows the decrease of $\overline{DP}_n$ versus the time depending on the catalyst exemplarily for OLG 4.

4. Conclusion

To investigate the relationship between oligomer molecular weight and polymer degradation, low molecular weight oligo(D,L-lactic acid)s of various molecular weights ($\overline{M}_n$ 290 to 1320 Da) have been synthesized and fully characterized.
The solubility of various polydisperse oligomers was determined via direct measurements. It was found that the solubility of oligomers depends on both pH and average molecular weight. Polydisperse oligomers with $\overline{M}_n$ smaller than 830 Da are soluble in buffer at pH 7.4 whereas oligomers of $\overline{M}_n \geq 830$ Da are insoluble. The kinetic studies showed that the hydrolysis rate of all tested oligomers was the slowest at pH 4.5. The energy of activation ranged from 47 to 67 kJ/ mol depending on average molecular weight of oligomers.
From the mechanistic study utilizing ^{1}H-NMR spectroscopy it could be conducted that the oligomer degradation mechanism is dependent on the pH of the surrounding medium. Under acidic conditions chain-end cleavage is the mode of action whereas under basic conditions degradation of oligomers occur via random ester cleavage and/ or backbiting.
Typically, in a medium of pH 7.4, the pH inside the bulk drops due to the formation of free carboxylic acid groups which cannot leach out the bulk. In contrast pH at the surface remains unchanged because of the buffer capacity and the exchange of buffer in the in vitro model. According to the theory of bulk degradation, hydrolysis should be faster in the inner part than at the surface due to the higher concentration of ester and carboxyl bonds resulting in the inner part in a lower pH value (the so-called “autocatalytic effect”).
Our results clearly demonstrate that hydrolysis slows down with decreasing pH values up to a pH of approximately 4.5 and once the pH is lower than 4.5 hydrolysis speeds up again. These results indicate that at least in the initial degradation stage the degradation was not dominated by the so-called “autocatalytic effect”.

Results generated in our studies demonstrate the need to investigate other factors than pH to better understand and explain the bulk degradation nature of PLA and PLGA since pH has proven to play only a minor role.

Acknowledgements

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References

[1] Kissel T, Traechslin E. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J Control Release 1992; 21: 129-138.

[2] Vert M, Suming L, Garreau H. More about the degradation of LA/GA-derived matrices in aqueous media. J Control Release 1991; 16: 15-26.

[3] Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 1997; 28 (1): 5-27.

[4] Vert M, Feijen J, Albertsson A, Scott G, Chiellini E, editors. Biodegradable polymers and plastics. Cambridge: Royal Soc Chem Spec Pub, 1992.

[5] Vert M, Li SM, Garreau H. Attempts to map the structure and degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. J Biomater Sci Polym Ed 1994; 6 (7): 639-649.

[6] Makino K, Ohshima H, Kondo T. Mechanism of hydrolytic degradation of poly(L-lactide) microcapsules: effect of pH, ionic strength and buffer concentration. J Microencapsulation 1986; 3 (3): 203-212.

[7] Espartero JL, Rashkov I, Li SM, Manolova N, Vert M. NMR Analysis of low molecular weight poly(lactic acid)s. Macromolecules 1996; 29: 3535-3539.

[8] Vert M, Schwach G, Engel R, Coudane J. Something new in the field of PLA/GA bioresorbable polymers? J Control Release 1998; 53 (1-3): 85-92.

[9] Vert M, Mauduit J, Li S. Biodegradation of PLA/GA polymers: increasing complexity. Biomaterials 1994; 15 (15): 1209-1213.

[10] Shah S, Cha Y, Pitt C. Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery ? J Control Release 1992; 18: 261-270.

[11] Li S, Vert M. Hydrolytic degradation of the coral/ poly(DL-lactic acid) bioresorbable material. J Biomater Sci Polym Ed 1996; 7 (9): 817-827.

[12] Li S, Vert M. Biodegradable Polymers: Polyesters. In: Mathiowitz E, editor. Encyclopedia of Controlled Drug Delivery. New York: Wiley John, 1999. p. 71-93.

[13] Therin M, Christel P, Li S, Garreau H, Vert M. In vivo degradation of massive poly(alpha-hydroxy acids): validation of in vitro findings. Biomaterials 1992; 13 (9): 594-600.

[14] Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 1995; 16 (4): 305-311.

[15] Avgoustakis K, Nixon JR. Biodegradable controlled tablets 3. Effect of polymer characteristics on drug release. Int J Pharm 1993; 99: 247-252.

[16] Shah S, Cha Y, Pitt C. Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery ? J Control Release 1992; 18: 261-270.

[17] von Burkersroda F, Gref R, Goepferich A. Erosion of biodegradable block copolymers made of poly(D,L-lactic acid) and poly(ethylene glycol). Biomaterials 1997; 18 (24): 1599-1607.

[18] Maniar M, Kalonia D, Simonelli A. Determination of specific rate constants of specific oligomers during polyester hydrolysis. J Pharm Sci 1991; 80 (8): 778-782.

[19] Maniar ML, Kalonia DS, Simonelli AP. Alkaline hydrolysis of oligomers of tartrate esters: effect of a neighboring carboxyl on the reactivity of ester groups. J Pharm Sci 1992; 81 (7): 705-709.

[20] Braud C, Devarieux R, Garreau H, Vert M. Capillary electrophoresis to analyze water-soluble oligo(hydroxyacids) issued from degraded or biodegraded aliphatic polyesters. J Environ Polym Degrad 1996; 4 (3): 135-148.

[21] Park TG. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. Biomaterials 1995; 16 (15): 1123-1130.

[22] Park TG. Degradation of poly(D,L-lactic acid) microspheres: effect of molecular weight. J Control Release 1994; 30: 161-173.

[23] Li SM, Garreau H, Vert M. Structure-property relationships in the case of the degradation of massive aliphatic poly-(alpha-hydroxyacids) in aqueous media, Part 1: Poly(D,L-lactic acid). J Mater Sci Mater Med 1990; 1: 123-130.

[24] Mauduit J, Perouse E, Vert M. Hydrolytic degradation of films prepared from blends of high and low molecular weight poly(DL-lactic acid)s. J Biomed Mater Res 1996; 30 (2): 201-207.

[25] deJong SJ, Arias EA, Rijkers DTS, van Nostrum CF, Kettenes van den Bosch JJ, Hennink WE. New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus. Polymer 2001; 42 (7): 2795-2802.

[26] Mauduit J, Bukh N, Vert M. Gentamycin/ poly(lactic acid) blends aimed at sustained release local antibiotic therapy administered per-operatively. I. The case of gentamycin base and gentamycin sulfat in poly(DL-lactic acid) oligomers. J Control Release 1993; 23: 209-220.

[27] Wang N, Wu XS, Lujan-Upton H. Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/ glycolic acid oligomers: 1. Synthesis and characterization. J Biomater Sci Polym Ed 1997; 8 (12): 905-917.

[28] Vidil C, Braud C, Garreau H, Vert M. Monitoring of the poly(D,L-lactic acid) degradation by-products by capillary zone electrophoresis. J Chromatography A 1995; 711: 323-329.

[29] Giunchedi P, Conti B, Scalia S, Conte U. In vitro degradation study of polyester microspheres by a new HPLC method for monomer release determination. J Control Release 1998; 56 (1-3): 53-62.

[30] Braud C, Devarieux R, Atlan A, Ducos C, Vert M. Capillary zone electrophoresis in normal or reverse polarity separation modes for the analysis of hydroxy acid oligomers in neutral phosphate buffer. J Chromatography B 1998; 706 (1): 73-82.

[31] Chen J, Gardella JA. Time-of-flight secondary ion mass spectrometry studies of in vitro hydrolytic degradation of biodegradable polymers. Macromolecules 1999; 32: 7380-7388.

[32] deJong S, van Dijk-Wolthuis WNE, Kettenes van den Bosch JJ, Schuyl PJ, Hennink WE. Monodisperse Enantiomeric Lactic Acid Oligomers: Preparation, Characterization, and Stereocomplex Formation. Macromolecules 1998; 31 (19): 6397-6402.

[33] Ramchandani M, Pankaskie M, Robinson D. The influence of manufacturing procedure on the degradation of poly(lactide-co-glycolide) 85:15 and 50:50 implants. J Control Release 1997; 43 (2-3): 161-173.

[34] Shih C. Chain-end scission in acid catalyzed hydrolysis of poly(D,L-lactide) in solution. J Control Release 1995; 34: 9-15.

[35] Shih C. A graphical method for the determination of the mode of hydrolysis of biodegradable polymers. Pharm Res 1995; 12 (12): 2036-2040.

[36] Witzke DR, Narayan R. Reversible Kinetics and Thermodynamics of the Homopolymerization of L-Lactide with 2-Ethylhexanoic Acid Tin(II) Salt. Macromolecules 1997; 30 (23): 7075-7085.

[37] Goepferich A. Mechanism of polymer degradation and elimination. In: Domb AJ, Kost J, Wiseman DM, editors. Handbook of biodegradable polymers. Amsterdam: Harwood Academic Publishers, 1997. p. 451-471.

[38] Dunne M, Corrigan OI, Ramtoola Z. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. Biomaterials 2000; 21 (16): 1659-1668.

[39] Rafler G, Jobmann M. Controlled Release Systems of Biodegradable Polymers 1st Communication: Hydrolytic degradation of biodegradable aliphatic polyesters. Pharm Ind 1994; 56 (6): 565-570.

[40] Witt C. PhD thesis, Marburg, 2000.

[41] Ronneberger B. PhD thesis, Marburg, 1995.

Chapter 3

Hydrolytic degradation of poly(lactide-co-glycolide) films:

Effect of oligomers on degradation rate and crystallinity

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Abstract

Oligomers are thought to accelerate the hydrolytic degradation of devices prepared from poly(lactide-co-glycolide), PLGA, due to their increased number of carboxylic end groups compared to polymer. To experimentally verify this hypothesis, two D,L-lactic acid oligomers having molecular weights close to their critical limit of solubility were synthesized and incorporated into PLGA films in three concentrations (0, 10 and 30 % w/ w).

All films were translucent, rather flexible and initially amorphous. With increasing oligomer concentration the glass transition temperature (T_g) and the molecular weight of films decreased prior to erosion.

The degradation studies show that initial mass loss and water absorption are increased in oligomer-containing films as a function of average molecular weight and oligomer concentration. However, the incorporation of oligomers does not accelerate the degradation of films. By contrast, oligomer-containing films show extended lag-phase until onset of polymer erosion. This was shown to be related to crystallization as observed in parallel. Moreover, it was found that crystallization occurs earlier in oligomer-containing films and that the degree of crystallization is related to the average molecular weight of the oligomer. These findings bring new insight into the role of oligomers in the degradation process and can be used to explain why erosion in massive polymer devices occurs from the center to the surface.

1. Introduction

Poly(lactide-co-glycolide) (PLGA) is one of the most frequently studied class of biodegradable polymers, especially for controlled delivery of peptides [1, 2] and proteins [3, 4]. The major advantage of these polymers is that they do not require surgical removal after completion of drug release.

The degradation of aliphatic polyesters has been investigated by numerous authors. It is generally accepted that PLGA, and their homopolymers polylactic acid (PLA) and polyglycolic acid (PGA) degrade via bulk hydrolysis of ester bonds [5, 6, 7]. Finally, their constituent monomers lactic and glycolic acid are formed which are eliminated by metabolic pathways [8]. It has been shown that the degradation rate is affected by several physical and chemical factors, such as initial pH, ionic strength and temperature of external medium, copolymer ratio, molecular weight, crystallinity and specimen size [9, 10, 11].

However, until now the degradation process has not been completely understood. From a general point of view, two phenomena are discussed. Firstly, degradation causes an increase of the number of carboxylic end groups, which are known to autocatalyze ester hydrolysis [12, 13, 14]. Secondly, with increasing degradation time the amount of oligomer within the polymer matrix increases and soluble oligomers can escape from the whole mass of device. In larger specimens only soluble oligomers which are located close to the surface can diffuse from the matrix before they are totally degraded, whereas oligomers located more inside the matrix remain entrapped and increase the acidity within the matrix. The encapsulated oligomers increase the concentration of ester and carboxyl bonds, which results in an increased degradation rate and autocatalysis with respect to the outer part of the specimen. These diffusion-reaction phenomenona [15, 16, 17] lead to a differentiation between surface and center in larger specimen [7, 9, 18]. Recently new parameters have been identified which contribute to the bulk erosion process [19, 20].

Although oligomers play an important role in the complex bulk degradation mechanism only few authors have studied their influence on degradation kinetics [21, 22]. However, the molecular weight of low molecular weight polymers studied was far away from their critical limit of solubility [23, 24]. Due to the fact that this parameter is important with respect to their effect on polymer degradation [25, 26]

only limited information can be obtained from previous studies about the role of oligomers in this process.

For this reason a series of D,L-lactic acid oligomers were synthesized and characterized with regard to solubility, degradation rate and degradation mechanism [26]. Two oligomers having average molecular weights close to their critical limit of solubility were selected and incorporated into PLGA films in different concentrations (0, 10 and 30 % w/ w).

The intention of the present study was to test the hypothesis that oligomers autocatalyze the degradation process [12, 13, 14]. If oligomers increase the polymer degradation rate the lifetime of the oligomer-containing PLGA films should be shorter than the lifetime of the oligomer-free PLGA film.

In consideration of the fact that until now crystallization caused by PLGA degradation has never been investigated as a function of the oligomer, the main interest was focused on this issue.

2. Experimentals

2.1 Materials

D,L-lactic acid oligomers (OLA) were synthesized by polycondensation of 90 % D,L-lactic acid aqueous solution without any catalyst as described previously [26]. Briefly, 90 % D,L-lactic acid aqueous solution was allowed to concentrate by gentle distillation of water. The reaction started at normal pressure and was then changed to reduced pressure after removal of water. The temperature was slowly increased to reach 140 °C after 3 days. The polydisperse oligomers obtained had weight average molecular weights ($\overline{M}_w$) of 1700 Da (OLA-1) and 3200 Da (OLA-2).

Uncapped poly(lactide-co-glycolide) (PLGA), Type Resomer® RG 503H, lactide/ glycolide ratio 50:50, $\overline{M}_w$ 27.4 kDa, was purchased from Boehringer Ingelheim, Ingelheim, Germany. All organic solvents were HPLC grade and provided from Merck, Darmstadt, Germany.

2.2 Preparation of films

All films were prepared by a solvent casting method. Briefly, 0, 10 or 30 % w/ w of OLA-1 or OLA-2 were added to PLGA and dissolved in 10 ml acetone. The solution

was poured into a Teflon® mould and the solvent was allowed to evaporated at 8 °C for 24 hours. The films were then dried under vacuum at room temperature to remove residual solvent until a constant weight was obtained. The resulting films had a thickness of about 200 - 250 µm and were cut into disks of 11-mm diameter using a punch. The final weight of films was 16.4 ± 0.96 mg (n = 30).

2.3 Determination of molecular weight

The average molecular weight was determined by size exclusion chromatography (SEC) using polystyrene standards (M_w 400 to $2.5 \cdot 10^6$ Da) (Polymer Standard Service, Mainz, Germany) for calibration. The samples were dissolved in tetrahydrofuran (THF) and filtered before injection. THF containing 0.1 % trifluoracetic acid (TFA) was used as mobile phase at a flow rate of 1 ml/ min. Two PSS® columns, 7.8 x 300 mm, with a pore size of 10^3 Å and 10^5 Å (Polymer Standard Service, Mainz, Germany) connected in series were used to separate sample fractions at 30 °C. A differential refractometer (ERC 7510, Tokyo, Japan) was used for detection. Each sample was analyzed in duplicate and data were processed using ChromStar 4.1® software (SCPA, Stuhr, Germany).

2.4 Determination of glass transition temperature

Measurement of glass transition temperature (T_g) was performed using a differential scanning calorimeter (DSC 821, Mettler Toledo, Greifensee, Switzerland). Two samples (4-7 mg) were heated twice under nitrogen atmosphere. Thermograms covering a range of -20 °C to 200 °C were recorded at a heating or cooling rate of 10 K/ min. Calibration of the system was performed using gallium and indium standards. The onset temperature, which corresponds to the temperature at which the signal first derives from baseline was used to describe the phase transition and was evaluated from the second heating run (STARe® software 6.0, Mettler Toledo, Greifensee, Switzerland).

2.5 X-ray diffraction (XRD)

X-ray diffraction patterns were recorded with an automatic powder diffractometer D 5000® (Siemens, Munich, Germany) using a CuK_α radiation source (40 kV, 30 mA) and a nickel filter (1.54 Å). The scanning speed was 0.2 degree/ min. The maximum

scattering angle (2θ) was 35°. Separate blank patterns were recorded to allow subtraction of air-scattering and sample holder. Sharp peaks or broad halos were observed in diffraction pattern of crystalline or amorphous film, respectively. Crystallinity was calculated using DiffracPlus 3.0® software (Bruker, Rheinstetten, Germany).

2.6 Degradation studies

Weighed film specimens were placed in previously weighed glass vials and immersed in 12 ml phosphate puffer (0.05 M, pH 7.4 containing 0.05 % benzalconium chloride and 0.1 % sodium azide). The samples were incubated at 37 °C for 4 weeks without agitation. Six parallel samples were tested for each type of film. The buffer solution was replaced after each sampling time in order to prevent pH changes due to polymer degradation. At different time-intervals, the films were removed, washed threefold with water and weighed after removal of surface water. The samples were then dried for at least 48 h in a lyophilizer.

Water absorption and mass loss were calculated using the following equations:

Water absorption (%) = 100 $(W_w-W_d)/ W_d$

Mass loss (%) = 100 $(W_0 -W_d)/ W_0$

where W_w and W_d represent the mass of film in wet and dry state, respectively.W_0 is the film weight determined initially.

SEC and DSC were applied to monitor the degradation of films whereas XRD was used to detect crystallinity in degraded polymer films.

3. Results and discussion

3.1 Physicochemical characteristics of PLGA/OLA films

Using the solvent-casting technique, five different PLGA films were produced. The properties of applied materials are listed in Table 1.

In order to clarify the miscibility of PLGA and D,L-lactic acid oligomer all films were analyzed by DSC. The presence of a single T_g in all blends confirmed that PLGA and oligo-D,L-lactides are miscible at all the given composition. Miscibility was also observed at higher oligomer concentrations, however, the mechanical strength of resulting films was insufficient.

All films were characterized prior to erosion and results are summarized in Table 2. The acronyms are reflecting the nature of each film matrix.

The addition of low molecular weight oligomers resulted in a decrease of molecular weight of the films produced.

Table 1. Characteristics of materials used

Code	Molecular weight (kDa)		$\overline{M_w}$ / $\overline{M_n}$	T_g (°C)
	$\overline{M_w}$	$\overline{M_n}$		
OLA-1	1.7	0.96	1.8	15.6
OLA-2	3.2	1.5	2.1	26.8
PLGA	27.4	16.0	1.7	44.5

Table 2. Compositions and characteristics of produced PLGA films

Code	Oligomer	Composition (%)		Molecular weight (kDa)		$\overline{M}_w$ / $\overline{M}_n$	T_g (°C)
		PLGA	OLA	$\overline{M}_w$	$\overline{M}_n$		
Reference	None	100	0	24.7	14.4	1.7	44.3
PLGA/OLA-1 10 %	OLA-1	90	10	19.6	5.6	3.5	40.7
PLGA/OLA-1 30 %	OLA-1	70	30	16.8	3.3	5.2	34.0
PLGA/OLA-2 10 %	OLA-2	90	10	20.9	7.1	2.9	42.6
PLGA/OLA-2 30 %	OLA-2	70	30	18.4	5.4	3.4	36.5

This results mainly from the mixing of different molecular weights and less from degradation caused by incorporated oligomers [21].

It was observed that the number average molecular weight ($\overline{M}_n$) of oligomer-containing films decreased more than $\overline{M}_w$. This can be explained by the fact that $\overline{M}_n$ depends more on the fraction of low molecular weight than on the fraction of high molecular weight.

In parallel the polydispersity index (PI) increased in oligomer-containing films. The PI is defined as the ratio of $\overline{M}_w$ to $\overline{M}_n$ and describes the broadness of molecular weight distribution within a polymer. From Table 2 it can be seen that PI is a function

of oligomer loading and of the average molecular weight of oligomer. The higher the oligomer loading and the lower $\overline{M}_w$ of oligomer the higher PI of film.

3.2 In vitro degradation profiles

The films listed in Table 2 were incubated in phosphate buffer pH 7.4 at 37 °C. As degradation parameters water absorption, mass loss (both gravimetrically) and weight loss (SEC) were monitored. Morphological changes were detected by DSC and X-ray diffraction.

3.2.1 Visual examination

All prepared films were initially translucent and elastic depending on average molecular weight and percentage of oligomer in the film matrix. After one of day incubation all films were white and no longer translucent. With increasing incubation time the diameter of all films increased dramatically. After freeze-drying all films were waxy-like, wavy and brittle.

3.2.2 Water absorption and glass transition temperature

Water absorption was detected from beginning of incubation in buffer (Fig. 1a). The initial amount of absorbed water was a function of average molecular weight of oligomer and their percentage in PLGA film. Water absorption of all films increased steadily with time because degradation causes an increase in polymer hydrophilicity. The incorporation of oligomers increases the hydrophilicity of the film due to their hydroxylic and carboxylic end groups. In consequence, oligomer-containing films absorbed more water than the oligomer-free film.

For PLGA/OLA-1 10 and 30 % the amount of water absorbed after 3 days was 186 and 240 %, respectively, compared to 41 % in oligomer-free film as reference (Fig. 1a). However, after 5 days water absorption was higher in the PLGA/OLA-1 10 % film than in PLGA/OLA-1 30% film. We assume that with an increasing amount of oligomer incorporated the leaching out of OLA-1 increases and consequently the hydrophilicity of the remaining film decreases.

On the other hand with rising oligomer chain length the hydrophilicity of oligomer decreased due to its increased number of hydrophobic methyl groups. Thus more time is needed to degrade the oligomer in a soluble state. In consequence degradation of

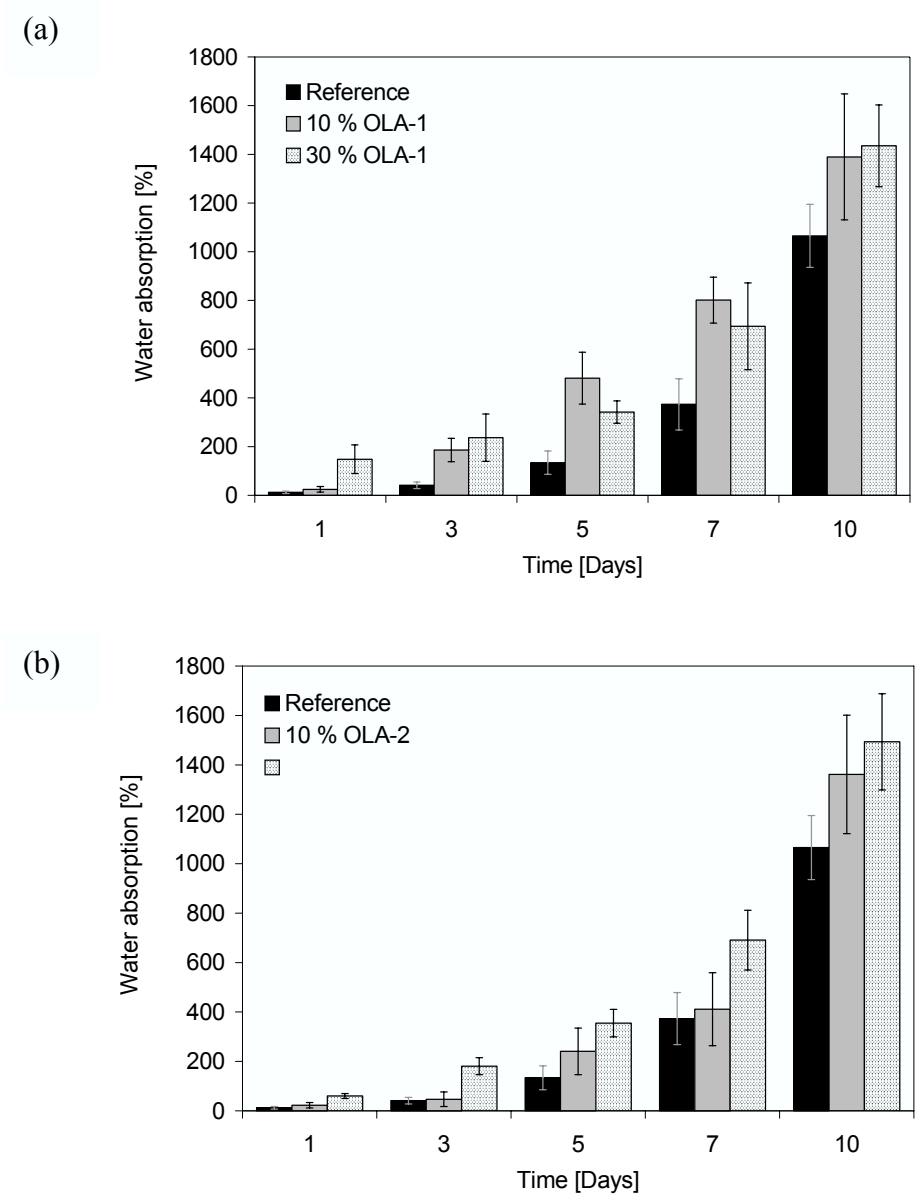

Figure 1. Effect of addition of oligomers (0, 10 and 30 %) on water absorption of PLGA 50:50 films during in vitro degradation: (a) for OLA-1 and (b) for OLA-2. The five types of films are presented in Table 2

polymers of the same chemical composition but different molecular weights is slower for the higher molecular weight compound [27].

Comparing OLA-1 and OLA-2 the first has a lower average molecular weight and glass transition temperature whereas the latter is more lipophilic. Therefore initial water uptake was reduced in films containing OLA-2. For PLGA/OLA-2 10 and 30 % the amount of water absorbed after 3 days was only 47 and 180 %, respectively (Fig. 1b).

Table 3 Glass transition temperature of degraded PLGA films

	T_g (°C)							
Sample	0 d	3 d	5 d	7 d	10 d	14 d	18 d	21 d
PLGA	44.3	40.7	39.2	37.2	35.1	36.3	41.7	45.5
PLGA/OLA-1 10%	40.7	39.0	37.6	35.3	33.9	41.1	46.2	46.2
PLGA/OLA-1 30%	34.2	40.3	37.2	34.6	33.8	29.7	44.4	40.5
PLGA/OLA-2 10%	42.6	38.5	37.7	36.6	35.6	31.9	43.9	39.1
PLGA/OLA-2 30%	36.5	39.7	38.5	38.0	36.3	38.3	41.7	40.2

As expected water absorption of PLGA/OLA-2 films was a function of oligomer loading over the whole time period.

In parallel to water absorption the glass transition temperature of films was monitored as a function of oligomer molecular weight and oligomer loading during degradation (Table 3). It is known that a decrease of $\overline{M}_W$ leads to a decrease of T_g which is attributed to an easier chain mobility in polymers [17]. From Table 3 it can be seen that T_g decreased directly after incubation in buffer with one exception due to polymer degradation and water absorption [25, 28]. For PLGA films, containing 30 % OLA-1 or OLA-2 an increase of T_g was observed after 3 days. This was caused by diffusion of a major part of the incorporated oligomers out of the film. The remaining higher molecular weight fraction is characterized by a higher T_g as known from literature [29].

Surprisingly T_g started to increase in all films after 10 or 14 days and reached values greater than those in the beginning [30]. In parallel with the observed increase of T_g an endothermic peak was detected in the first run of DSC experiment (Fig. 2). This was assigned to the melting of crystalline domains formed during degradation [15, 30]. X-ray diffraction was successfully used to confirm this assumption. All results demonstrated that incorporation of oligomers in PLGA film clearly enhanced their hydrophilicity [31].

3.2.3 Crystallinity

In all films no crystallinity was found at the beginning of degradation studies. The XRD pattern of initial films is exemplary shown for PLGA/OLA-2 10 % (Fig. 3a).

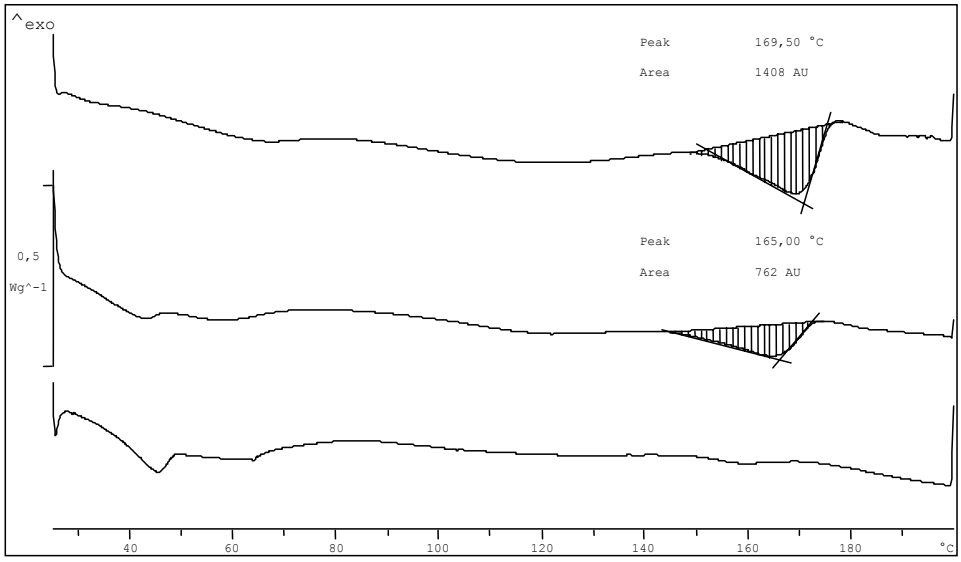

Figure 2. DSC thermograms obtained after 14 days immersion in buffer pH 7.4: (a) PLGA/OLA-1 10 %, (b) PLGA/OLA-2 10 % and (c) reference. AU means area units.

The obtained halo pattern is typical for an amorphous polymeric compound and confirmed that all films were initially amorphous.

However, after 2 weeks of incubation crystallinity was detected in 10 % oligomer-containing films indicating that oligomers contribute to morphological changes during degradation. This can be explained by the fact that with increasing water content, molecule chains in the polymer matrix become more flexible and mobile enough to crystallize under such conditions [29, 32].

It was found that the degree of crystallinity was higher in films containing OLA-1 ($\overline{M}_w$ 1700 Da) instead of OLA-2 ($\overline{M}_w$ 3200 Da) (Table 4). Based on this finding we conclude that the degree of crystallinity at this time point was depending on the average molecular weight of oligomer added. With increasing incubation time crystallization occurred also in reference and 30 % oligomer-containing films. The diffraction pattern of oligomer-free and oligomer-containing films obtained after 18 days of incubation are shown in Fig. 3b and Fig. 3c. Five sharp peaks were detected (2θ = 16.9, 18.9, 21.9, 27.7 and 32.6°) which demonstrated the presence of crystalline domains within all polymer films. As expected crystallinity was higher in oligomer-containing films confirming our hypothesis that oligomers affect directly or indirectly crystallization during degradation (Table 4).

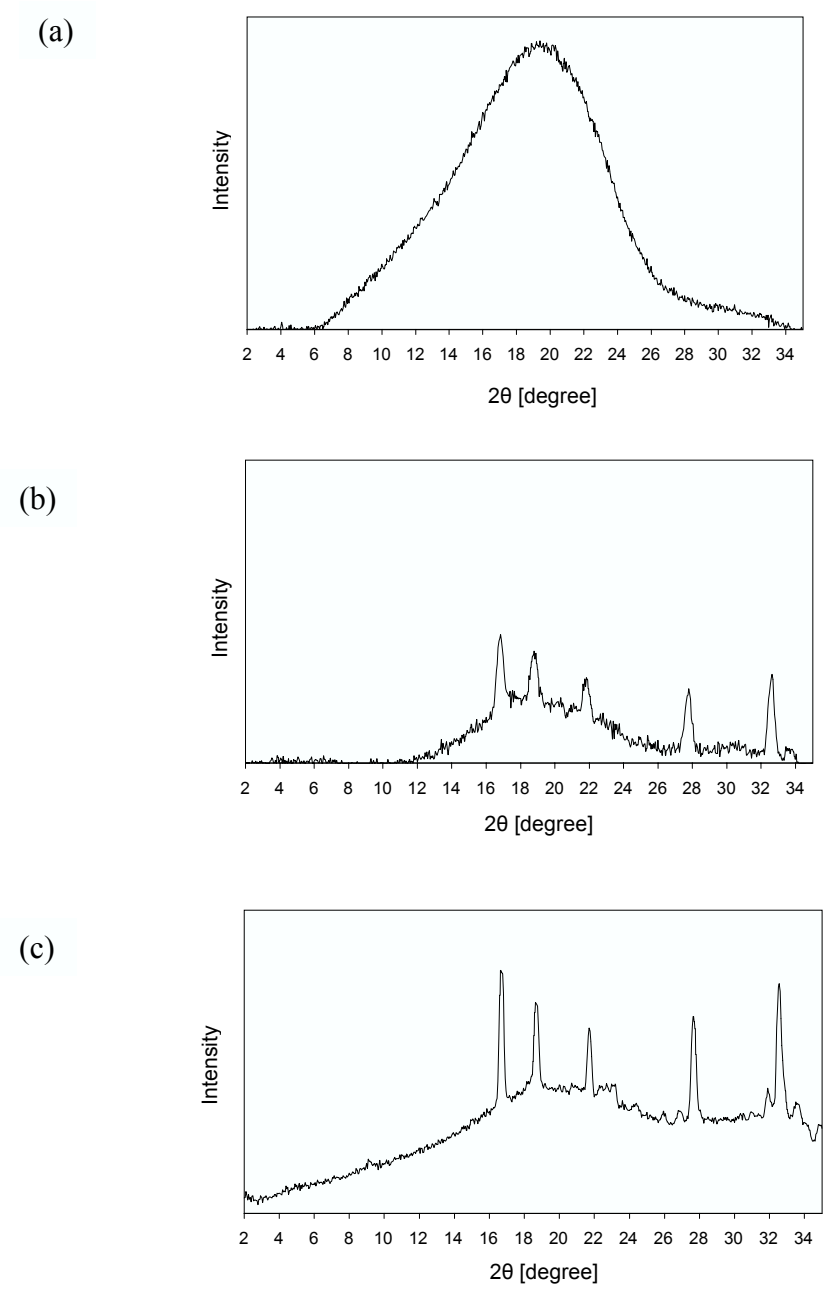

Figure 3. X-ray diffraction pattern obtained for (a) PLGA/OLA-1 10 % before immersion, (b) reference and (c) PLGA/OLA-1 10 % after 18 days immersion in buffer pH 7.4.

Unfortunately, after 3 weeks no X-ray pattern could be obtained due to the very small amounts of residual polymer mass.

The results show that the degree of crystallinity in oligomer-containing films depends on average molecular weight and percentage of incorporated oligomer. One can hypothesize that this finding is related to the differences in T_g and molecular weight

Table 4 Crystallinity of degraded PLGA films

Code	Crystallinity (%)			
	0 d	10 d	14 d	18 d
Reference	0	0	0	22
PLGA/OLA-1 10 %	0	0	26	28
PLGA/OLA-1 30 %	0	0	0	34
PLGA/OLA-2 10 %	0	0	15	31
PLGA/OLA-2 30 %	0	0	0	20

between the two oligomers. These should influence the chain mobility and the reorganization of chains within the film [33].

However, crystallization was only observed at later stages of polymer degradation. The driving force for such morphological change is a closer packing of polymer chains with consequent enhancement of intermolecular attraction [29].

3.2.4 Mass loss and molecular weight changes

The $\overline{M}_w$ of oligomer-free film decreased with time (Fig. 4). In contrast $\overline{M}_w$ of oligomer-containing film was either unchanged (10 % OLA) or increased (30 % OLA) within the first 24 hours. Due to the higher average molecular weight of OLA-2 the increase of $\overline{M}_w$ observed was smaller than for OLA-1 containing films. Beyond this $\overline{M}_w$ of all blends decreased with time as expected and reached a plateau after 2 weeks. This can be explained by the fact that films degrading for longer than 10 days in buffer, were partially insoluble in THF and thus excluded from analysis. In addition degradation products, which are small enough to be soluble, diffused out from the film and were also excluded from the analysis. As a consequence no further decline of $\overline{M}_w$ was observed until the end of the study. However, the best representation of the molecular weight progression is the polydispersity index.

As shown in Fig. 5a, the PI (closed symbols) of PLGA/OLA-1 10 and 30 % decreased from 3.5 and 4.3 to 2.1 and 2.6, respectively within the first 3 days. In contrast PI of reference increased slightly within these first days indicating that a smaller molecular weight fraction was formed.

Mass loss of films (open symbols, Fig. 5) was observed immediately after incubation in buffer.

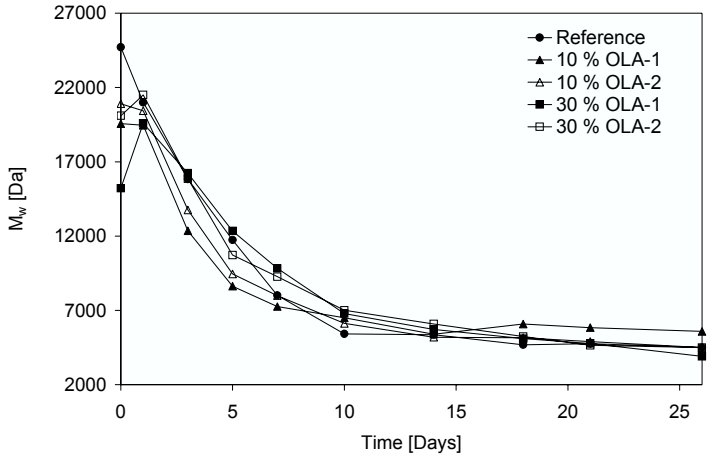

Figure 4. Degradation profiles of oligomer-free and oligomer-containing films which are presented in Table 2.

In general mass loss was higher for films containing OLA-1 due to its smaller $\overline{M}_w$ and consequently higher solubility. As expected initial mass loss increased with increasing amount of incorporated oligomer.

The initial mass losses were 8, 12 and 21 % for reference, PLGA/OLA-1 10 and 30 %, respectively (Fig. 5a). This can be assigned to the release of both residual acetone and incorporated oligomer. The initial mass loss observed, as well as the reduction of PI, confirmed our assumption that a great amount of low molecular weight fraction had left the film matrix within 3 days.

No remarkable mass loss was detected until the end of the week. After 10 days mass loss had reached 22, 24 and 30 % compared to 15, 14 and 21 % after 1 week for reference, PLGA/OLA-1 10 and 30 %. The PI of these films decreased in combination with a loss of $\overline{M}_w$, as shown in Fig. 4., indicating that most of the higher molecular weight fraction and incorporated oligomer were degraded and had partially left the film matrix. After 14 days mass loss of oligomer-free film was accelerated in comparison to 18 days found for PLGA/OLA-1 10 and 30 % films.

In Fig. 5b mass loss and changes in molar weight distribution of PLGA/OLA-2 10 and 30 % are shown. No differences were found between initial mass loss of reference and PLGA/OLA-2 10 % compared to a slightly increased mass loss of PLGA/OLA-2 30 % during the first 5 days. In parallel PI decreased from 2.95 and 3.45 to 2.3 and 2.5 for PLGA/OLA-2 10 and 30 %, respectively. Mass loss increased after 1 week from

10 and 17 % to 25 and 32 % after 10 days for PLGA/OLA-2 10 and 30 %, respectively, and was rather constant until day 18. From then on mass loss of PLGA/OLA-2 10 and 30 % was accelerated which was also found for films containing OLA-1. At the end of the study the remaining mass of all films was less than 5 %.

In summary, for all oligomer-containing films a prolonged lag phase up to the onset of accelerated mass loss was observed. This observation leads to the assumption that the

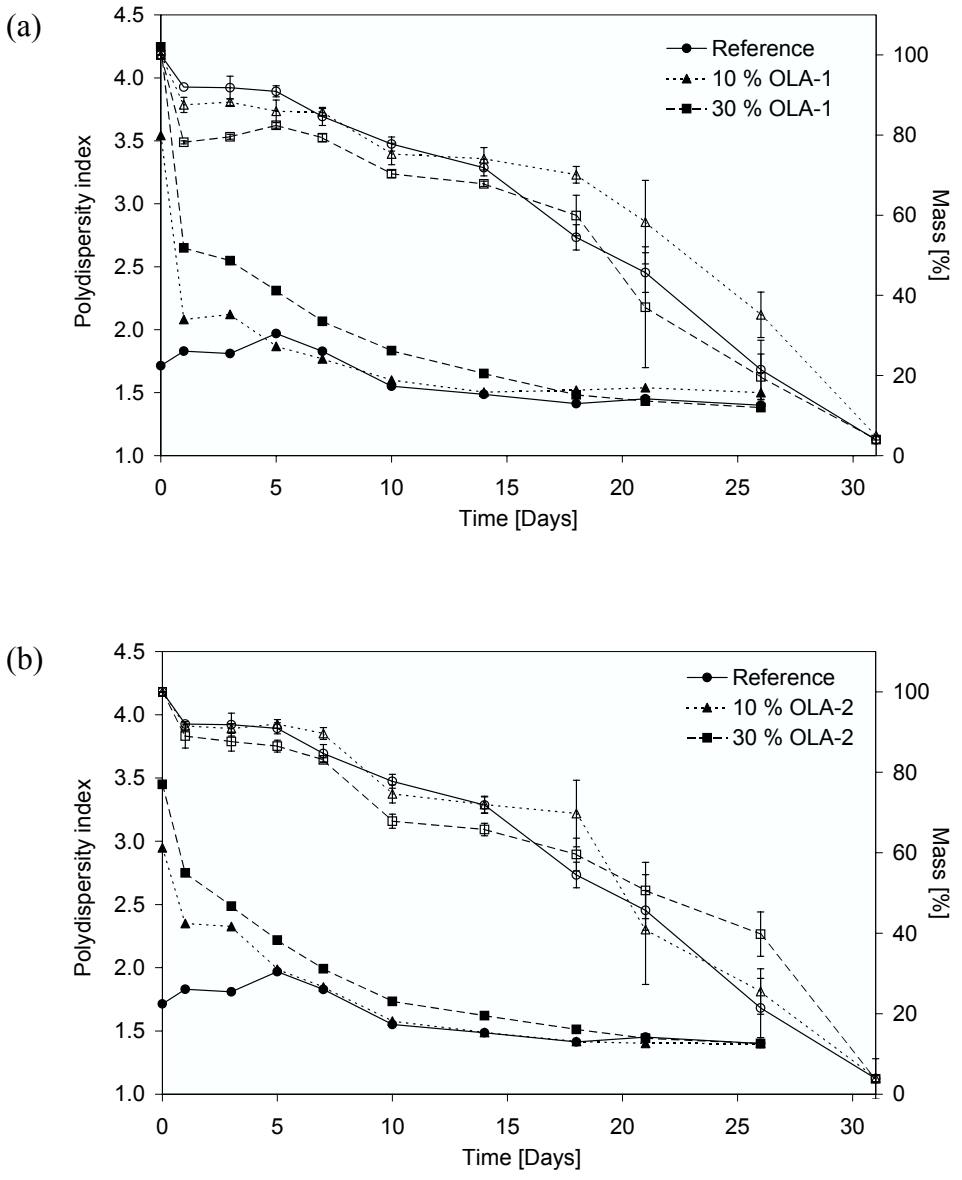

Figure 5 Effect of addition of oligomers (0, 10 and 30 %) on mass loss (open symbols) and polydispersity (closed symbols) of PLGA films: (a) PLGA/OLA-1 and (b) PLGA/OLA-2.

during of lag phase is influenced by oligomers. The extent to which degradation is affected depends on both the average molecular weight of the oligomer incorporated and the amount of remaining oligomer.

4. Conclusion

The influence of the average molecular weight and the concentration of D,L-lactic acid oligomers added on the degradation rate and crystallinity of PLGA 50:50 film was investigated. The incorporation of polydisperse oligomers clearly enhanced the hydrophilicity of PLGA film. The initial mass loss and the amount of water absorbed were functions of average molecular weight and concentration of oligomer. For the same oligomer initial mass loss and water uptake was enhanced with increasing amount of oligomer in the film. However, an autocatalytic effect caused by the increased number of carboxylic end groups due to the incorporation of oligomers was not observed.

The degradation studies point to the fact that all initially amorphous polymer films changed into semi-crystalline films. Moreover, it was found that oligomers contribute to such morphological change due to their properties like low T_g, short chain length, low $\overline{M}_w$ and hydrophilicity that facilitate crystallization. It was found that the time until crystallization occurred, as well as the degree of crystallization, depends on the average molecular weight of oligomers added and their remaining concentration in the film.

During this study no differentiation between surface and center was observed due to the small specimen size. However, the finding that oligomers cause direct or indirect crystallization during degradation of an initially amorphous PLGA matrix can be used to explain the surface/ center differentiation in large specimen in accordance with described diffusion-reaction phenomenona. In further studies the influence of oligomers on degradation rate and crystallinity in massive polymer devices will be assessed to test this hypothesis. A challenge for the future will be to visualize the distribution of oligomers incorporated during the degradation process.

References

[1] Blanco-Preto MJ, Besseghir K, Zerbe O, Andris D, Orsolini P, Heimgartner F, Merkle HP, Gander B. In vitro and in vivo evaluation of a somatostatin analogue released from PLGA microspheres. J Control Release 2000; 67, (1): 29-36.

[2] Cleland JL, Johnson OL, Putney S, Jones AJS. Recombinant human growth hormone poly(lactic-co-glycolic acid) microsphere formulation development. Adv Drug Deliv Rev 1997; 28, (1): 71-84.

[3] Bittner B, Witt C, Maeder K, Kissel T. Degradation and protein release properties of microspheres prepared from biodegradable poly(lactide-co-glycolide) and ABA triblock copolymers: influence of buffer media on polymer erosion and bovine serum albumin release. J Control Release 1999; 60, (2-3): 297-309.

[4] Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm Res 1991; 8, (6): 713-720.

[5] Avgoustakis K, Nixon, JR. Biodegradable controlled tablets 3. Effect of polymer characteristics on drug release. Int. J. Pharm. 99 (1993) 247-252.

[6] Kenley RA, Lee MO, Mahoney TR, Sanders LM. Poly(lactide-co-glycolide) decomposition kinetics in vivo and in vitro. Macromolecules 1987; 20, (10): 2398-2403.

[7] Li S, Garreau H, Vert M. Structure-property relationships in the case of the degradation of massive aliphatic poly-(alpha-hydroxyacids) in aqueous media, Part 2: Degradation of lactide-glycolide copolymers: PLA37.5GA25 and PLA75GA25. J Mater Sci Mater Med 1990; 1: 131-139.

[8] Wu XS. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: Wise D, editor. Encyclopedic Handbook of Biomaterials and Bioengineering. New York: Marcel Dekker, 1995. pp. 1015-1054.

[9] Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 1995; 16, (4): 305-311.

[10] Makino K, Ohshima H, Kondo T. Mechanism of hydrolytic degradation of poly(L-lactide) microcapsules: effect of pH, ionic strength and buffer concentration. J Microencapsulation 1986; 3, (3): 203-212.

[11] Miyajima M, Koshika A, Okada J, Ikeda M, Nishimura K. Effect of polymer crystallinity on papaverine release from poly (lactic acid) matrix. J Control Release 1997; 49, (2-3): 207-215.

[12] Pitt CG. Non-microbial degradation of polyesters: mechanism and modifications. In: Vert M, Feijen J, Albertsson A, Scott G, Chiellini E. editors. Biodegradable polymers and plastics Cambridge: Royal Society of Chemistry, 1992. pp. 7-19.

[13] Shah S, Cha Y, Pitt C. Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery ? J Control Release 1992; 18: 261-270.

[14] Vert M, Li SM, Garreau H. Attempts to map the structure and degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. J Biomater Sci Polym Ed 1994; 6, (7): 639-649.

[15] Therin M, Christel P, Li S, Garreau H, Vert M. In vivo degradation of massive poly(alpha-hydroxy acids): validation of in vitro findings. Biomaterials 1992; 13, (9): 594-600.

[16] Vert M, Schwach G, Engel R, Coudane J. Something new in the field of PLA/GA bioresorbable polymers? J Control Release 1998; 53, (1-3): 85-92.

[17] Avgoustakis K, Nixon JR. Biodegradable controlled tablets 1. Preparative variables affecting the properties of poly(lactide-co-glycolide) copolymers as matrix forming material. Int J Pharm 1991; 70: 77-85.

[18] Li S, Garreau H, Vert M. Structure-property relationships in the case of the degradation of massive aliphatic poly-(alpha-hydroxyacids) in aqueous media, Part 1: Poly(D,L-lactic acid). J Mater Sci Mater Med 1990; 1: 123-130.

[19] deJong SJ, Arias EA, Rijkers DTS, van Nostrum CF, Kettenes-van den Bosch JJ, Hennink WE. New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus. Polymer 2001; 42, (7): 2795-2802.

[20] von Burkersroda F, Schedl L, Göpferich A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 2002; 23, (21): 4221-4231.

[21] Mauduit J, Perouse E, Vert M. Hydrolytic degradation of films prepared from blends of high and low molecular weight poly(DL-lactic acid)s. J Biomed Mater Res 1996; 30, (2): 201-207.

[22] Grandfils C, Flandroy P, Jérôme R. Control of the biodegradation rate of poly(L-lactide) microparticles intended as chemoembolization materials. J Control Release 1996; 38, (2-3): 109-122.

[23] Vidil C, Braud C, Garreau H, Vert M. Monitoring of the poly(D,L-lactic acid) degradation by-products by capillary zone electrohoresis. J Chromatography A 1995; 711: 323-329.

[24] Braud C, Devarieux R, Garreau H, Vert M. Capillary electrophoresis to analyze water-soluble oligo(hydroxyacids) issued from degraded or biodegraded aliphatic polyesters. J Environ Polym Degrad 1996; 4, (3): 135-148.

[25] Vert M, Mauduit J, Li S. Biodegradation of PLA/GA polymers: increasing complexity. Biomaterials 1994; 15, (15): 1209-1213.

[26] Schliecker G, Schmidt C, Fuchs S, Kissel T. Characterization of a homologous series of D,L-lactic acid oligomers: A mechanistic study on the degradation kinetics in vitro. Biomaterials 2003, 24: 3835-3844.

[27] Belbella A, Vauthier C, Fessi H, Devissaguet JP, Puisieux F. In vitro degradation of nanospheres from poly(D,L-lactides) of different molecular weights and polydispersities. Int J Pharm 1996; 129: 95-102.

[28] Bodmeier R, Oh KH, Chen H. The effect of the addition of low molecular weight poly(D,L-lactide) on drug relese from biodegradable poly(D,L-lactide) drug delivery systems. Int J Pharm 1989; 51: 1-8.

[29] Heller J. Fundamentals of Polymer Science. In: Robinson, JR., Lee, VHL, editors. Controlled Drug Delivery Fundamentals and Applications. New York: Marcel Dekker, 1987. pp. 139-212.

[30] Seo S-A, Choi HS, Gilson K, Rhee JM, Lee HB. A local delivery system for fentanyl based on biodegradable poly(lactide-co-glycolide) oligomer. Inter J Pharm 2002; 239, (1-2): 93-101.

[31] Geze A, Venier-Julienne MC, Saulnier P, Varlet P, Daumas-Duport C, Devauchelle P, Benoit JP. Modulated release of IdUrd from poly (d,l-lactide-co-glycolide) microspheres by addition of poly (d,l-lactide) oligomers. J Control Release 1999; 58, (3): 311-322.

[32] Li S, Vert M. Biodegradable Polymers: Polyesters. In: Mathiowitz E. editor. Encyclopedia of Controlled Drug Delivery. New York: Wiley John 1999, pp. 71-93.

[33] Goepferich A. Mechanism of polymer degradation and elimination. In: Domb A, Kost J, Wiseman D, editors. Handbook of biodegradable polymers. Amsterdam: Harwood Academic Publishers, 1998. pp. 451-471.

[34] Lewis DH, Controlled release of bioactive agents from lactide/glycolide polymers. In: Langer R, Chasin M, editors. Biodegradable polymers as drug delivery systems. New York: Marcel Dekker, 1990. pp. 1-41.

Chapter 4

Characterization and in vitro degradation of Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate)

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Abstract

In the present study, a less known polyester based on tartaric acid was characterized with respect to its degradation mechanism. Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (PTA) differs from commonly used biodegradable polyesters, such as poly(lactides-co-glycolides) (PLGA) by the presence of additional cleavable bonds in the polymer side chains

This modification results in different polymer properties and influences polymer degradation. The hydrolytic degradation of PTA was studied in parallel to PLGA using disc-shape matrices which were obtained by compression-molding. The discs were incubated in pH 7.4 phosphate buffer solution at 37 °C. The degraded samples were characterized for percentage mass loss, water absorption, decay of molecular weight and change in glass transition temperature. The results demonstrate that the degradation of PTA proceeds via bulk erosion similar to PLGA. However, the degradation of PTA implants is characterized by a rapid mass loss within a short period of time appearing after a definite lag phase without remarkable mass loss. This makes the polymer promising for pulsatile drug release systems.

1. Introduction

Biodegradable polymers have gained importance in the development of controlled release devices. A number of biodegradable polymers have been synthesized and evaluated for their potential use as drug delivery system. Major interest in this area has focused on aliphatic polyesters such as polylactide-co-glycolides (PLGA) and their homopolymers due to the favorable toxicology of their degradation products [1]. The degradation of these polymers occurs via hydrolytic ester bond cleavage, leading to a decrease in their molecular weight [2-5]. The molecular architecture of the polymer influences among other things the in vitro degradation properties which should be related to the release properties of the polymer.

In the present study, the degradation of a less known tartaric acid-based polyester (polytartrate) was studied. Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (PTA) is a branched polytartrate and was first described in 1991 [6, 7]. Early experiments using this polymer revealed the suitability of this material for preparation of microparticles and its suitability for controlled release of peptides [8, 9]. In contrast to PLGA having all hydrolytic sensitive ester bonds located in the polymer main chain, PTA contains additionally ester as well as ketal groups in the polymer side chains. This affects the properties of PTA especially the degradation velocity which determines the type of polymer erosion.

In general, polymer erosion can be classified into homogeneous or *bulk erosion*, and heterogeneous or *surface erosion* [10]. However, it should be mentioned that both are extremes and that the erosion mechanism of a polymer device is often characterized by a combination of both.

Surface eroding polymers contain e.g. highly labile bonds (polyanhydride) or acid-labile bonds (polyorthoesters) in their polymer main chain, which degrade very fast in contact with water. Thus the degradation rate on the surface is much faster than the penetration rate of water into the polymer bulk. In consequence erosion is limited to the surface and drug release is degradation controlled [11]. This behavior changes if degradation rate is slower than the rate of water diffusion into the device. In this case water penetrates into the bulk and degradation occurs throughout the polymer (*bulk erosion*). The resulting pores and channels in the matrix influences the rates of polymer degradation and drug release [12]. Therefore, predicting and controlling the

release kinetics of drugs from bulk eroding polymers like PLGA is very difficult and surface eroding polymers are preferred.

The aim of this study was to investigate the degradation behavior of PTA with respect to its degradation mechanism, which has not been published before. Since studying the effect of device size on degradation rate can provide insight into the degradation mechanism of polymer [13, 14] three different sizes of PTA disc-shaped matrices were examined to determine if primarily surface or bulk erosion occured. The hydrolytic degradation in pH 7.4 phosphate buffer solution was monitored by molecular weight decay, mass loss, water absorption, change in glass transition temperature and monomer release. Finally the degradation and mechanism of PTA and PLGA matrices of identical size were investigated in a comparative study.

2. Materials and Methods

2.1 Materials

Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (PTA) was received from Aventis R&T, Frankfurt, Germany. The molecular structure is shown in Fig. 1. Poly(D,L-lactide-co-glycolide) (PLGA), copolymer ratio 50:50, Type Resomer®RG502H was purchased from Boehringer Ingelheim, Ingelheim, Germany. Tetrahydrofuran (THF), trifluoroacetic acid (TFA), methylene chloride, acetonitrile-d_3 (ACN-d_3), and the buffer chemicals were purchased from Merck, Darmstadt, Germany.

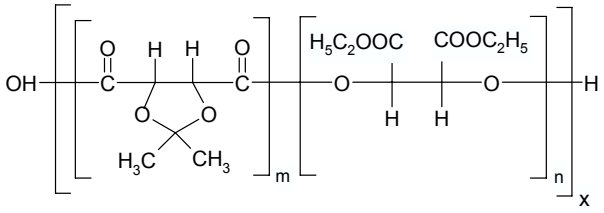

Figure 1. Structure of PTA

2.2 Characterization of polymer

2.2.1 ^{1}H-NMR

^{1}H-NMR spectra of PTA were recorded on a Bruker spectrometer operating at 500 MHz at room temperature (16 scans). 20 mg of polymer was dissolved in ACN-d_3, filtrated and transferred into a NMR tube (approximately 1.8 ml) to determine

polymer composition. Chemical shifts (δ) were expressed in ppm with respect to the ACN-d_3 signal at 1.96 ppm: CH_3 -triplet at 1.26 ppm, CH_3 -singulet at 1.45 ppm, CH_2 –quadruplet at 4.2 ppm, CH –singulet at 5.05 ppm and CH –singulet at 5.82 ppm [15].

2.2.2 Size exclusion chromatography (SEC)

The molecular weight was determined by size exclusion chromatography using twostyragel columns (PSS guard column SDV, PSS SDV 100, 5 µm, PSS SDV linear M, 10 µm, 300 x 7.5 mm, Polymer Standard Service, Mainz, Germany). Degassed THF containing 0.1 % (v/ v) trifluoroacetic acid (TFA) was used as mobile phase at a flow rate of 1 ml/ min. A polymer aliquot (n=3) was dissolved in THF (20 mg/ ml) and filtrated before injection. The weight average molecular weight ($\overline{M}_w$) and the number average molecular weight ($\overline{M}_n$) were calculated relative to polystyrene standards (Polymer Standard Service, Mainz, Germany) using refractive index detection (ERC 7510, Tokyo, Japan) at 30 °C. Each sample was analyzed in duplicate and data were processed using ChromStar 4.1® software (SCPA, Stuhr, Germany).

2.2.3 Differential scanning calorimetry (DSC)

Measurement of glass transition temperature (T_g) was performed using a differential scanning calorimeter (DSC 821, Mettler Toledo, Greifensee, CH). Two samples (~ 7 mg) of polymer were heated twice under nitrogen atmosphere. Thermograms covering a range from – 60 to 200 °C were recorded at a heating and cooling rate of 10 K/ min. Calibration of the system was performed using gallium and indium standards. The onset temperature which corresponds to the temperature at which the signal first derives from baseline was used to describe the phase transition and was evaluated from the second heating run (STARe® software 6.0, Mettler Toledo, Greifensee, Switzerland).

2.3 Preparation of polymer matrices

Disc-shaped matrices were prepared by compression molding at room temperature under appropriate pressure using a single punch excenter press (EK 0, Korsch Berlin, Germany) instrumented with a strain gauge for compression force measurements (Catman® 2.1, Hottinger Baldwin Messtechnik, Darmstadt, Germany). The polymer powder (size ≤ 500 µm) was compressed using two flat-faced punches of 3, 5 or 7

mm in diameter. The powder for each disc was weighed on an analytical balance and manually filled into the die. The compaction load (maximum upper punch pressure) was adjusted such that the same force was applied for each disc. The thickness of the discs obtained was 1.57±0.07 mm and the weight was 14, 40 or 86 mg, respectively.

2.4 In vitro degradation

The weighed implants were placed into weighed glass vials and 12 ml phosphate buffer (0.05 M, pH 7.4 containing 0.05 % benzalkonium chloride and 0.1 % sodium azide) were added. Samples were incubated at 37 °C without agitation for 4 weeks and occasionally shaken (twice/week) for 15 seconds. After various time-periods four implants were removed, washed with demineralized water and lyophilized. The buffer solution was replaced after each sampling in order to prevent pH changes due to polymer degradation. The degree of polymer degradation was measured by SEC and DSC. Mass loss (ML) and water absorption (WA) were determined gravimetrically using the following equations:

(1) $ML\ (\%) = 100 \times (W_0 - W_d)/ W_0$

(2) $WA\ (\%) = 100 \times (W_w - W_d)/ W_d$

where W_0 is the implant weight determined initially, W_w is the wet weight of implant and W_d the weight of implant after lyophilization.

2.5 Monomer release

PTA discs (n = 3), 7 mm in diameter, were placed into vials, 6 ml phosphate buffer (0.05 M, pH 7.4 containing 0.05 % benzalkonium chloride and 0.1 % sodium azide) were added and the vials were sealed with a crimping cap. Samples were incubated at 37 °C without agitation as described previously. At predetermined time points the medium was completely removed with a syringe and transferred into an empty sealed crimping vial. Fresh medium was added to the sample through the vial septum using a syringe. The removed medium was analyzed for the amount of monomeric degradation products. The concentration of L-tartaric acid released in the medium was measured by HPLC (Waters 2690, Waters Corporation, Milford, M.A.). Separation was performed on a C18 column (225 x 4 mm I.D., Machery Nagel, Düren, Germany) at 30 °C. Sulfuric acid solution of pH 2.5 was used as mobile phase at a flow rate of 0.35 ml/ min [16]. Detection was performed using a Waters 2470 programmable

absorbance detector (Waters Corporation, Milford, USA) operating at 215 nm. Calibration curves were generated from known concentrations of L-tartaric acid in release medium. The detection limit was 1 µg/ ml. The Millenium 32® chromatography manager, version 4.0 (Waters Corporation, Milford, USA) was used to analyze the data.

The concentration of acetone and ethanol in the release medium was analyzed using a gas chromatography system equipped with a flame ionization detector attached to a Headspace sampler (HP 5890, Hewlett Packard, Germany). The temperatures were selected as follows: oven temperature 80 °C, injector temperature 150 °C and detector temperature 200 °C. The chromatograph was fitted with a 50 m x 0.32 mm i.d. CP-SIL 5CB column coated with 5 µm film of stationary phase (Chrompack, Middleburg, The Netherlands) [17].

2.6 Photomicrography of PTA matrices

The degradation of PTA matrices was visualized using a photo camera (EOS 30, Canon, Germany) adapted with a 70-300 mm lens (Voigtländer, Fürth, Germany) for macroscopic view. Additionally lyophilized polymer matrices were investigated microscopically (Axioplan stereo microscope, Zeiss, Jena, Germany) before and after degradation. Photographs of the surfaces and cross-sections of polymer matrix were recorded using a digital microscope camera (DP 10, Olympus, Hamburg, Germany).

3. Results and discussion

3.1 Polymer characteristics

The representative ^{1}H-NMR spectrum of poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) is shown in Fig. 2 and the structure of the polymer was confirmed by the peak assignments. The ^{1}H-NMR signals of 2,3-isopropylidentartrat (monomer 1) were found at 1.45 ppm (-C(C$\underline{H}_3$)$_2$) and at 5.05 ppm (-CH). The signals of 1,3-diethyltartrat (monomer 2) resonated at 1.26 ppm (-CH$_2$C$\underline{H}_3$), at 4.2 ppm (-C$\underline{H}_2$CH$_3$) and 5.82 ppm (-CH). The molar composition was determined by comparing the methyl signal of monomer 1 at 1.45 ppm and the methylene signal of monomer 2 at 1.26 ppm and calculated as 50 to 50. As consequence of the polycondensation the series of monomers is strictly alternating.

Figure 2. ^{1}H-NMR spectra and structure of poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (PTA).

The average molecular weight of PTA calculated from polystyrene standards was found to be 23 kDa. The polymer is amorphous and characterized by a glass transition temperature of 41.9 °C. The thermal properties of PTA are similar to PLGA 50:50 (T_g 40.5°C). Solubility studies demonstrated that PTA is soluble e.g. in acetone, THF, methylene chloride and acetonitrile but insoluble in water, petroleum ether and ethanol.

3.2 In vitro degradation

3.2.1 Influence of matrix size on in vitro degradation

In order to determine the influence of matrix size on degradation rate, PTA discs of three different diameters 3, 5 and 7 mm, were examined in buffer at pH 7.4.

It was hypothesized that if pure surface erosion occurs degradation is limited to the outer surface and degradation time is directly proportional to device size. In contrast, if bulk erosion occurs degradation is independent from device size leading to similar degradation rates [18].

The effect of matrix size on water absorption and molecular weight decay is shown in Fig. 3a. Due to the lipophilic methyl and ethyl groups in the polymer side chains the polymer is hydrophobic and hence no water can be absorbed initially. The measured

water uptake of less than 6 % w/w immediately after incubation in buffer results from penetration of water into the pores of the device. As known the polymer particles undergo plastic deformation under compression and the porosity of the matrix depends on the densification of the polymer particles decreasing with increasing compression force [19]. Since the matrices were compressed at high compaction load below the glass transition temperature of the applied polymer it is likely that the implant porosity is only a few percents. By weighting out the same amount of polymer and applying the same compaction load a reproducible porosity of discs can be assumed.

The absorption of water increases obviously after 7 days for 3 mm discs and after 10 days for 5 and 7 mm discs. This observation can be explained by the fact that hydrolysis occurs within the polymer matrix forming free carboxylic and hydroxylic end groups which increase the hydrophilicity of the polymer. It was found that the absorption rate was a function of matrix size increasing with decreasing matrix size. This finding is related to the specific surface of polymer matrices which decreases with increasing disc diameter.

Although only small amounts of water were absorbed initially the $\overline{M}_w$ of all polymer matrices decreased linear with time without any lag phase and independent from disc size. This proves that degradation proceeds immediately after incubation in buffer. After 2 weeks the $\overline{M}_w$ of polymer matrices was dramatically decreased to values below 2000 Da. We assume that during degradation soluble oligomers were formed which are able to diffuse out of the polymer matrix which is already known for PLGA and PLA matrices [ ].

The mass loss profile as function of matrix size is shown in Fig. 3b. The erosion of PTA discs occurred after a definite lag phase although the molecular weight decay was observed from the beginning. The onset time of mass loss increased slightly with increasing matrix size. However, the rate of mass loss was similar for all discs of different size, indicating the predominance of bulk erosion. In general mass loss occurred in parallel with an increase in water absorption. As seen from Fig. 3b the molecular weight at this time point dropped below 2000 Da and confirms our assumption that at this time degradation products became soluble and diffused out of the polymer matrix into the release medium leading to the mass loss of polymer matrix. It is interesting to note that PTA matrices lost a great amount of their mass

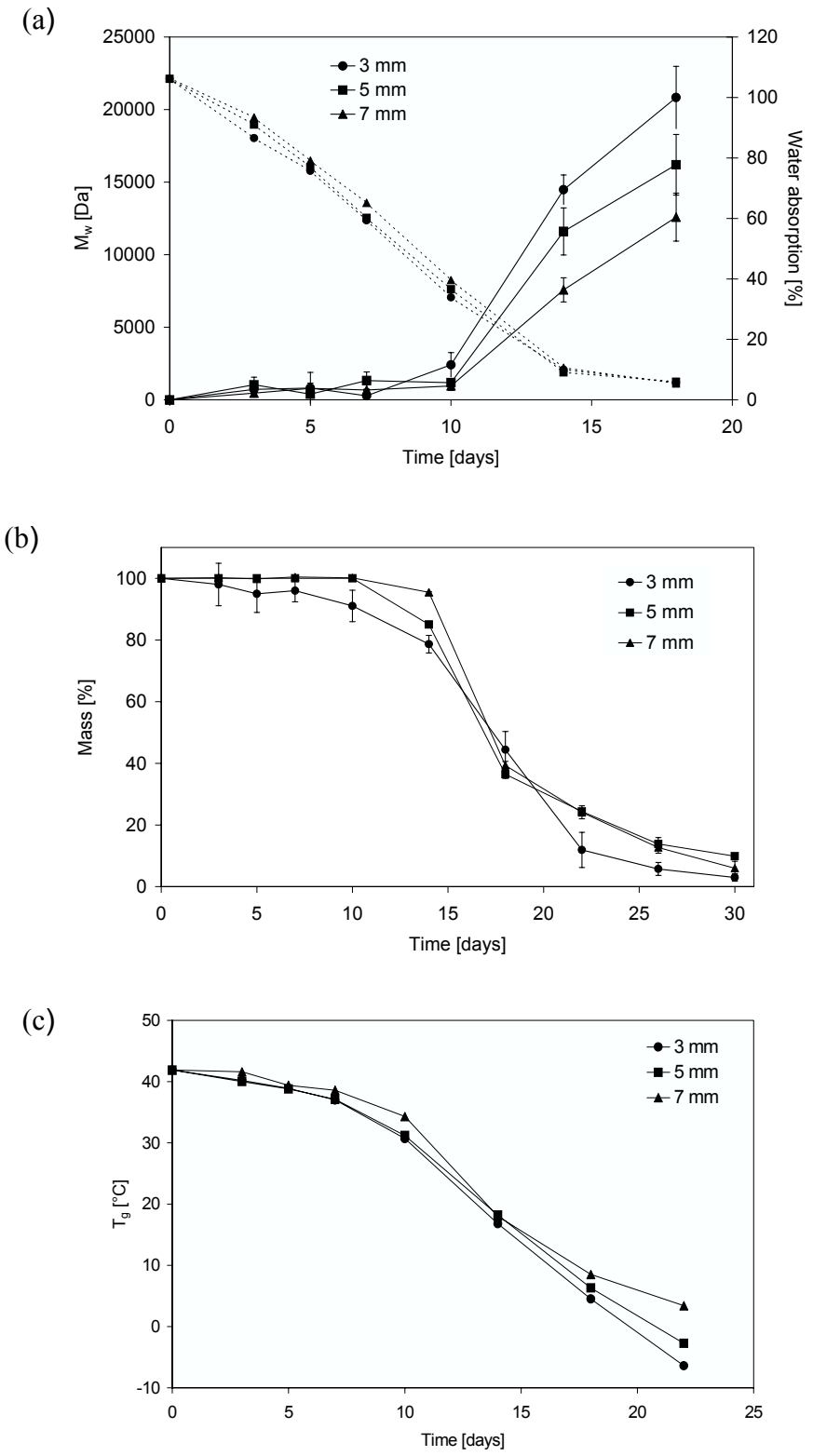

Figure 3. In vitro degradation of PTA matrices in buffer at pH 7.4: Effect of matrix size on water absorption (a) molecular weight decay (a), mass loss (b) and glass transition temperature (c).

within a short period of time. The remaining polymer mass eroded afterwards completely at a slower rate until the end of the study.

Additionally to water uptake, molecular weight decay and mass loss, the glass transition temperature was monitored during degradation (Fig. 3c). During the first week the glass transition temperature decreased slowly independent from matrix size due to the decrease in molecular weight. In parallel with an increase of water absorption and molecular weight decay T_g dropped fast and rather constantly to small values until the end of the study. The decrease of T_g is a result of both, absorption of water and formation of low molecular weight degradation products.

3.2.2 Monomer release

In order to gain more information about the degradation of PTA the release of the final degradation products tartaric acid, acetone and ethanol was monitored. The differential release profiles (Fig. 4) show an initial period of 10 days where only small amounts (< 1 ppm) of ethanol and acetone were released (Fig. 4, insert). The concentration of tartaric acid in the release medium was up to this time point below the limit of detection (< 1 µg/ml). The fact that only few amounts of monomeric degradation products were found initially is attributed to the time required for hydrolysis to produce sufficient amounts of monomers.

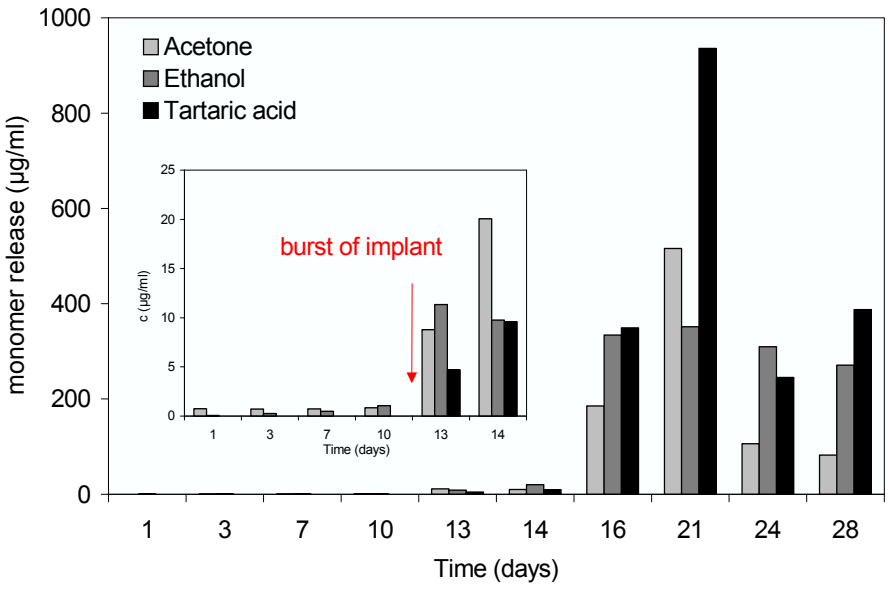

Figure 4. In vitro degradation of PTA: release of acetone, ethanol and L-tartaric acid from PTA matrices, 7 mm diameter

In parallel with an increase in water absorption the amount of tartaric acid, acetone and ethanol in the release medium started to raise. The concentration of tartaric acid, ethanol and acetone increased slowly on day 13 and 14 but thereafter a remarkable increase of monomers in the release medium was observed. The highest concentration of acetone and tartaric acid were found at day 21. It can be seen that the amount of ethanol released after defined time intervals was in general higher than those of acetone with exception of day 21. It can be assumed that a great amount of the ketal bonds were already cleaved at this time whereas some diethylester bonds were still unhydrolyzed. When comparing these results with the mass loss profile it can be seen that the onset time of mass loss correlates with the onset time of monomer release. Furthermore, the rate of monomer release was the highest from day 14 until day 21 which is in accordance with the rate of the mass loss at this time period.

3.2.3 Photomicrography of polymer matrices

In order to visualize the degradation process of PTA matrices the discs were observed and photographed during the degradation study (Fig. 5-7).

Immediately after immersion of polymer matrix in buffer a lot of bubbles were observed at the device surface which disappeared rather completely within 1 week (Fig. 5 a, b). As seen clearly, the polymer matrix did not swell until day 10 but thereafter in parallel with remarkable increase of water absorption the shape of the polymer matrix changed dramatically from a flat disc to a bloated convex device (Fig. 5c, d). Within few days the polymer shell bursted (Fig. 5e) and soluble degradation

Figure 5. Photomicrographs of the PTA disc during in vitro degradation: after (a) 3 days (b) 7 days, (c) 10 days, (d) 11days, (e)14 days (f) 18 days, (g) 21 days and (h) 26 days.

Figure 6. Surface-center differentiation of PTA matrices after degradation in buffer pH 7.4. Left column: cross section of lyophilized samples, right column: surface of lyophilized samples. From top to bottom: after 0, 3, 5, 7 and 10 days.

products such as oligomers and monomers were released into the medium as evidenced by the monomer release results which are in accordance with the onset of mass loss. The remaining polymer mass collapsed, resulting in a round device of reduced polymer mass, and degraded completely until week 4 (Fig. 5 f-h).

To obtain information about internal morphological changes the polymer matrix was removed at various time intervals, lyophilized and sectioned (Fig. 6). Additionally, the interest was focused on the surface characteristic of degraded matrix. Prior to erosion the matrix surface appeared slightly rough and less porous.

The cross section of the polymer matrix shows that the internal structure is also dense and homogenous (Fig. 6 a, b). After 3 days immersion in buffer, bubbles and blisters not present at the beginning are visible on the surface of the device (Fig. 6 d). Some of these blisters become to holes during the first week (Fig. 6 f, h) which were then closed likely by swelling at day 10. It can be seen that the surface is very smooth and appeared translucent at this time point (Fig. 6 k). Interestingly, the cross section of polymer matrix at day 3 shows a transparent glassy core and a white intransparent outer region (Fig. 6 c). Furthermore, it can be seen that the glassy core contains

Figure 7. Burst of PTA matrix during degradation: (a) image of lyophilized sample at day 13 and magnification of matrix shell (zoom)

Figure 8. Photomicrographs of PTA matrix obtained in buffer at day 3 (a, c) and after adding 50 µl of a acetone-containing buffer solution on the surface of the implant (b, d)

several air inclusions whereas only few pores are present close to the surface (Fig. 6 c-i).

The differentiation between surface and center became more obvious with increasing incubation time. The thickness of the white polymer layer decreased whereas the part of glassy polymer inside the polymer matrix increased (Fig. 6 c-i). Unfortunately it was no longer possible to observe the polymer matrices microscopically due to the burst of device between day 12 and 14. However, photographs of a polymer matrix lacerating during lyophilization show internal a glassy, swollen polymer mass and a surrounding thin white shell (Fig. 7).

As shown previously ethanol, acetone and tartaric acid are degradation products whose concentrations raise during degradation. However, only acetone is able to dissolve the polymer and induce surface changes as observed during degradation of polymer matrices (Fig. 8).

Based on all findings we hypothesis that the degradation of PTA discs proceeds as follows: After immersion of polymer matrix in buffer only small amounts of water penetrate into the device due to the low porosity of the polymer matrix and the initially hydrophobic character of the polymer. As a consequence of water penetration into the pores of device hydrolysis is not limited to the surface and occurs also in

polymer bulk. However, the penetration of water into device pores is strongly affected by mean pore size and pore size distribution which depends on compression force during matrix preparation [20, 21]. Due to the inhomogeneity of compression process the density distribution in the disc-shaped device is heterogeneous [22-24]. Furthermore, as the polymer has a relatively low glass transition temperature it is likely that particles which were in direct contact to the compression tool were sintered as consequence of friction energy occurring during compression and ejection of device [19, 25]. Therefore it is assumed that after placing the polymer matrix in buffer the amount of absorbed water in the center of the device is higher than near the surface. However, already small amounts of water have a noticeable plasticising effect leading to a reduction of the glass transition temperature of polymer [26]. This effect explains the observation that already after 3 days a glassy core was found inside the PTA discs. Bubbles located within the glassy core result from air embedded in the polymer matrix during the compression process.

With increasing degradation time the part of glassy polymer region increases most likely due to the ongoing hydrolysis that causes an increase in acetone and ethanol which act as plasticizer. In parallel, the molecular weight decreases and the hydrophilicity of polymer raises due to the formation of free hydroxylic and carboxylic end groups. As consequence the polymer is able to absorb water and thus short polymer chains become flexible. The resulting swelling of polymer causes the change in device shape. However, based on the assumption that the border areas of polymer matrix were sintered due to friction energy during ejection of device horizontal swelling is more complicated. This could explain the bloating of polymer matrix. With increasing degradation time the amount of small chain fragments increases and the resulting inner pressure causes the burst of implant shell between day 12 and 14. The enclosed soluble degradation products diffuse into the surrounding medium and mass loss occurs rapidly. The remaining insoluble polymer mass concentrates to reduce its surface area and degrades rather constantly until the end of the study.

3.2.4 Comparative study: PTA vs. PLGA

The aim of this study was to compare the erosion of PTA and PLGA disc-shaped matrices. One parameter of interest was the onset of polymer erosion depending on

polymer used for manufacturing of matrices. Due to the molecular architecture of the PTA it is likely that matrices made of PTA will differ in their erosion and degradation behavior compared to PLGA. In order to verify this assumption two batches of PTA and PLGA discs (code 1 and 2), 5 mm in diameter, were prepared under the same conditions. Since it is known that heat treatment of polymer matrices effect more the release characteristics than the degradation the discs were not tempered [27]. The in vitro degradation was evaluated separately and mass loss was taken as a measure for the erosion process.

For PLGA it is widely accepted that degradation in humid atmosphere occurs via random scission of ester linkages which are located exclusively in the polymer backbone. In general, the degradation process involves three steps: penetration of water into the device, secondly the hydrolysis of ester bonds in the polymer main chain leading to water soluble degradation products and the mass loss of the device through transport of degradation products into the surrounding medium. Due to the fact that PTA contains also cleavable bonds in the polymer side chains, degradation is not limited to the polymer main chain and occurs also in the side chains of polymer.

As a consequence of the hydrophobic character PTA matrices did not show a remarkable water absorption until day 10 whereas PLGA matrices started water absorption directly after immersion in buffer (Fig. 9a). It was found that PTA matrices absorb less water than PLGA matrices. Furthermore, the swelling behavior of the PTA matrix was different compared to PLGA. Whereas the first tended to increase

Table 1 Molecular weight decay of PTA and PLGA matrices, 5 mm in diameter, during incubation in buffer at pH 7.4

Time (weeks)	Molecular weight ($\overline{M}_w$) (Da)			
	PTA-1	PTA-2	PLGA-1	PLGA-2
0	23600	23000	10400	11000
1	13400	12950	4900	5300
2	2000	1950	3900	4250
3	1000	1000	3900	4100
4	< 1000	< 1000	4050	3950

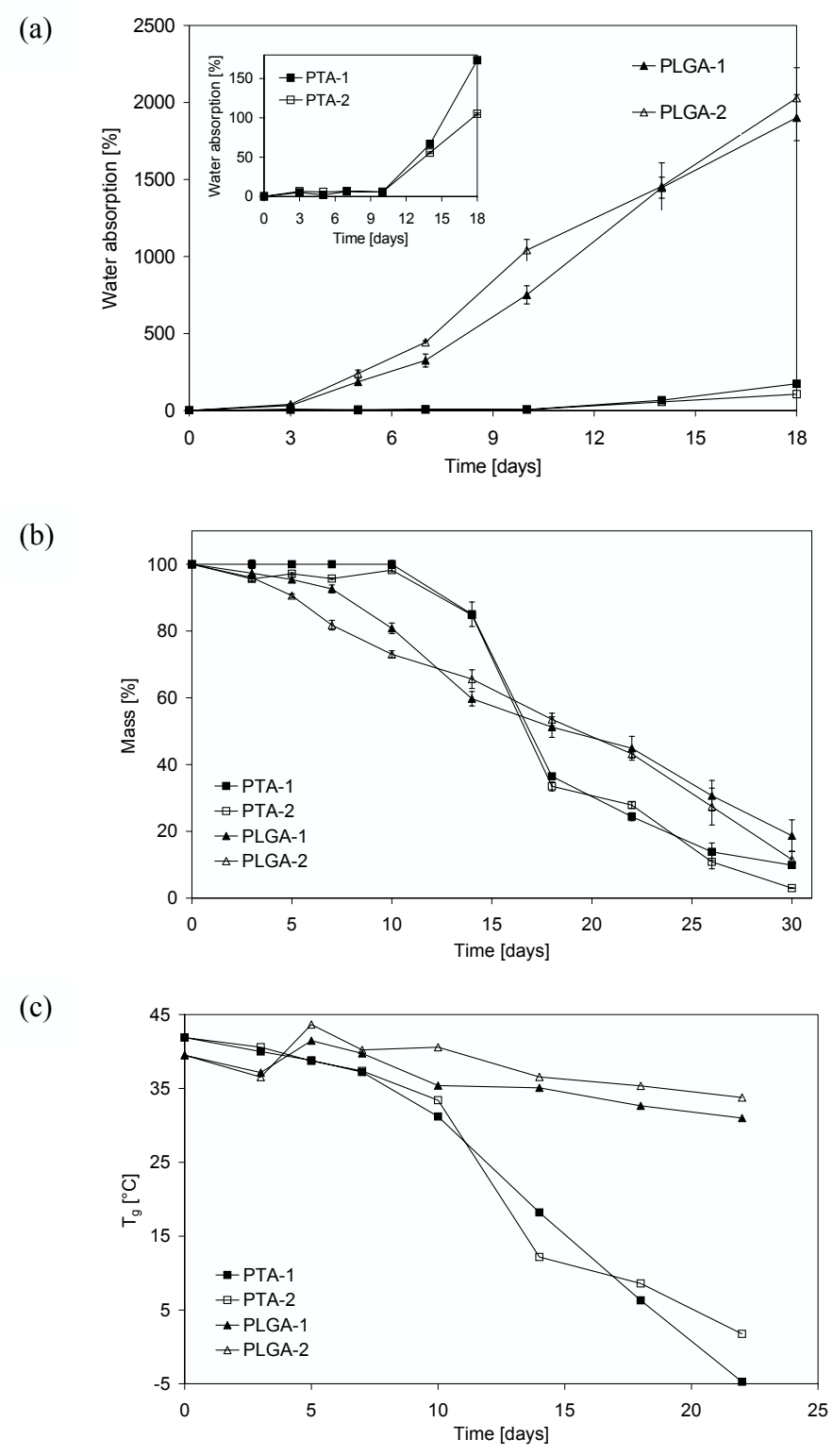

Figure 9. In vitro degradation of PTA and PLGA discs, 5mm in diameter (reproduceability study): water absorption (a), mass loss (b) and glass transition temperature (c).

more in height than in diameter the latter increased obviously in diameter and less in height.

The degradation of both polymers started without any lag phase during the first days and decreased linear with time (Table 1). The $\overline{M}_w$ of PLGA matrix reached a plateau after 2 weeks, which was related to two facts: Firstly, PLGA matrices which degraded longer than 10 days in buffer were partially insoluble in THF but only the soluble portion of degraded matrices could be analyzed. Secondly, from this time point small oligomers and monomers formed during degradation became mobile enough to diffuse out of the polymer matrix and were excluded from analysis. In consequence, no further decline of $\overline{M}_w$ was observed for PLGA matrices.

In contrast, all degraded PTA samples were soluble in THF and therefore degradation products with $\overline{M}_w > 1000$ Da could be detected in the samples.

As generally known the molecular weight has to be reduced substantially to permit mass loss through solubilization. Thus, mass loss is an index for the content of water-soluble degradation products.

The mass loss profiles of PTA and PLGA matrices are shown in Fig. 9 b. It can be seen that mass loss of PLGA matrices started directly after incubation in water and decreased linear with time. In contrast, no remarkable mass loss was observed for PTA matrices until day 10. Thereafter an accelerated mass loss was observed. The mass of polymer matrix decreased from 98 % at day 10 to 33 % at day 18. (Fig. 9 b). The remaining polymer mass degraded rather constantly until the end of the study.

Finally, the glass transition temperature was monitored as index for polymer degradation and water absorption. It can be seen in Fig. 9 c that T_g of PLGA discs increased initially which can be assigned to the release of low molecular weight compounds. Thereafter T_g declined slowly reaching values near 30 °C after 3 weeks. A different behavior was observed for PTA discs. The T_g of PTA discs declined slowly from the beginning until day 10. Thereafter the glass transition temperature dropped very fast to small values (< 5 °C). This can be assigned to degradation products like acetone and ethanol which act as plasticizer. It is also possible that oligomeric degradation products which are characterized by a very low T_g contribute to the decrease of T_g.

4. Conclusion

Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate), a less known biodegradable polyester was characterized in vitro. The degradation of polymer matrices was investigated in order to determine the erosion mechanism of of this polymer.

Acetone, ethanol and tartaric acid were identified as final degradation products which proves that degradation occurs in parallel in the polymer main chain and in the polymer side chains. During degradation the initially hydrophobic polymer changed into a hydrophilic one thus water absorption occurs subsequent to polymer hydrolysis. The results show that under the tested conditions PTA matrices undergo bulk erosion rather than surface erosion similar to PLGA matrices which were investigated in parallel.

During degradation a differentiation between surface and center was observed as described for massive devices made of PLGA and PLA. However, it has been shown that the degradation profile of PTA is different to those obtained from PLGA matrices. Surprisingly, the degradation of PTA matrices was characterized by a phase of rapid mass loss which appeared after a definite lag phase without any mass loss. This feature makes this polymer promising for pulsatile release and further studies are necessary to investigate drug release from PTA matrices.

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References

[1] Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Deliv. Rev. 28 (1997) 5-27.

[2] Li S, Vert M. Biodegradable Polymers: Polyesters. in: Mathiowitz E (Ed.), Encyclopedia of Controlled Drug Delivery. Wiley John, New York, (1999), pp. 71-93.

[3] Vert M, Schwach G, Engel R, Coudane J. Something new in the field of PLA/GA bioresorbable polymers? J. Control. Rel. 53 (1998) 85-92.

[4] Vert M, Mauduit J, Li S. Biodegradation of PLA/GA polymers: increasing complexity. Biomaterials 15 (1994) 1209-1213.

[5] Vert M, Suming L, Garreau H. More about the degradation of LA/GA-derived matrices in aqueous media. J. Control. Rel. 16 (1991) 15-26.

[6] Bengs H, Bayer U, Krone V, Lill N, Sandow J, Walch A. Polytartrate-a new biodegradable polymer. Proc. Int. Symp. ControI. Release Bioact. Mater. 23 (1996) 114-115.

[7] Ahlers M, Krone V, Walch A. Microparticles from biodegradable polymers. Adv. Mater. 4 (1992) 230-234.

[8] Krone V, Magerstädt M, Schrinner E, Walch A. DE-OS 3937283, Deutschland, 1991.

[9] Goepferich A, Tessmar J. Polyanhydride degradation and erosion. Adv. Drug Deliv. Rev. 54 (2002) 911-931.

[10] Goepferich A. Mechanism of polymer degradation and elimination. in: Domb A, Kost J, Wiseman D (Eds.), Handbook of biodegradable polymers. Harwood Academic Publishers, Amsterdam, (1998), pp. 451-471.

[11] Lewis DH. Controlled release of bioactive agents from lactide/glycolide polymers. in: Chasin M Langer R. (Eds.), Biodegradable polymers as drug delivery systems. Marcel Dekker, New York, (1990), pp. 1-41.

[12] Krone V, Magerstädt M, Walch A, Ditzinger G, Lill N, Polykondensate die Weinsäure enthalten, Verfahren zu ihrer Herstellung und Verwendung derselben, Europäisches Patentamt, 0514790A2, 1992.

[13] Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 16 (1995) 305-311.

[14] Witt C, Kissel T. Morphological characterization of microspheres, films and implants prepared from poly(lactide-co-glycolide) and ABA triblock copolymers: Is the erosion controlled by degradation, swelling or diffusion? Eur. J. Pharm. Biopharm. 51 (2001) 171-181.

[15] Sandow J, Ramorelix microparticles, results of biopharmaceutical tests in rats: PLGA vs PTA, Hoechst AG, Frankfurt, 1996, pp. 1-4.

[16] Shui G, Peng Leong L. Separation and determination of organic acids and phenolic compounds in fruit juices and dricks by high-performance liquid chromatography. J. Chromatogr. A 977 (2002) 89-96.

[17] Camarasu CC, Mezei-Szüts M, Varga GB. Residual solvents determination in pharmaceutical products by GC-HS and GC-MS–SPME. J. Pharm. Biomed. Anal. 18 (1998) 623-638.

[18] Akbari H, D'Emanuele A, Attwood D. Effect of geometry on the erosion characteristics of polyanhydride matrices. Int. J. Pharm. 160 (1998) 83-89.

[19] Führer C. Interparticulate attraction mechanism. in: Alderborn G, Nystrom C. (Eds.), Pharmaceutical Powder Compaction Technology. Marcel Dekker, New York, (1996), pp. 1-15.

[20] Riippi M, Yliruusi J, Niskanen T, Kiesvaara J. Dependence between dissolution rate and porosity of compressed erythromycin acistrate tablets. Eur. J. Pharm. Biopharm. 46 (1998) 169-175.

[21] Juppo AM. Porosity parameters of lactose, glucose and mannitol tablets obtained by mercury porosimetry. Int. J. Pharm. 129 (1996) 1-12.

[22] Moe D, Rippie EG. Nondestructive Viscoelastic Analysis of Anisotropy in Compresses Tablets. J. Pharm. Science 86 (1997) 26-32.

[23] Vachon MG, Chulia D. The use of energy indices in estimating powder compaction functionality of mixtures in pharmaceutical tableting. Int. J. Pharm. 177 (1999) 183-200.

[24] Kuentz M, Leuenberger H. A new model for the hardness of a compacted particle system, applied to tablets of pharmaceutical polymers. Powder Technology 111 (2000) 145-153.

[25] Guo HX, Heinämäki J, Yliruusi J. Characterization of particle deformation during compression measured by confocal laser scanning microscopy. Int. J. Pharm. 186 (1999) 99-108.

[26] Siemann U. The influence of water on the glass transition of poly(D,L-lactic acid). Therm Acta 85 (1985) 513-516.

[27] W. Vogelhuber, P. Rotunno, E. Magni, A. Gazzaniga, T. Spruss, G. Bernhardt, A. Buschauer, A. Göpferich, Programmable biodegradable implants, J. Control. Rel. 73 (2001) 75-88.

Chapter 5

Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate): a polymer for pulsatile release systems ?

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Abstract

In this study a less well known, biodegradable polyester, poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate, (PTA) was investigated as pulsatile release agent.

PTA implants, containing the LH-RH analogue buserelin, were prepared by compression molding. The effect of drug loading, implant size and admixing of polymers or excipients on drug release was investigated in vitro (pH 7.4, 37 °C).

In vitro studies show a multiphasic release profile charaterized by an initial burst which is followed by a lag phase. Thereafter, in contrast to common polyesters like poly(lactide-co-glycolide) (PLGA) a great amount of drug is released within a short period of time (second burst). The remaining drug is released rather constantly until the polymer is totally degraded. It was found that the initial burst increased concurrently with drug loading in contrast to the second burst which increased with decreasing drug loading. Furthermore, it could be shown that the second burst can be modified or supressed by blending PTA with other polymers or excipients.

Based on our results PTA has been identified as promising biodegradable polymer for pulsatile drug release.

1. Introduction

For many drugs a pulsatile release system, where the drug is released in intervals is advantageous and desired. The development of such a delivery system is of considerable interest, e.g. for vaccines and hormones [1-3].

In general vaccines are administered as an initial injection of antigen followed by repeated injections ("booster shots") at least two or three times. This is necessary to re-expose the immune system to an antigen and to enhance the extend of an immune response [4].

However, especially in the animal health industry repeated administration of a drug is a challenge because the treatment of large or free ranging herds of animals, e.g. cattle or sheep, is both a logistical and economical problem. In addition, each administration stresses the animals, which increases their susceptibility to diseases and causes adverse side effects at the injection side such as fibrous reactions. For these reasons a delivery system that releases vaccines in a way that a second treatment for the booster immunization is not required ("single-shot vaccine"), offers numerous benefits and is therefore highly requested from the markets [5].

Based on the knowledge of hormone secretion the development of a pulsatile release device for hormones is also of growing interest in animal and human health. The release of hormones in a pulsatile manner mimics the physiological pattern of hormone secretion and can be used for example in veterinary field for animal reproduction and breeding due to control of estrus .

Since estrus is only a short period of sexual receptivity in female animals where ovulation and insemination takes place it is difficult to detect. Thus the pulsatile release of hormones could synchronize the estrus to make female animals fertilizable at a predetermined time schedule, which is beneficial for livestock producers and for breeders [6].

In the past, pulsatile drug release in animals was achieved with implanted mechanical pumps or non-degradable controlled release systems [3]. At present particulate delivery systems based on biodegradable polymers, like poly(D,L-lactide-co-glycolide) (PLGA) or poly(ε-caprolactone) have been investigated as delivery system for vaccines and hormones. A single administration of different microspheres caused a pulsatile-like release profile. The release profile is adjustable via molecular weight,

polymer type, copolymer ratio and particle size of applied microspheres. Due to their different degradation rate drug is released at different time points [7-10].

However, microspheres do not release the booster dose as a sharp bolus, which is required especially for effective immunization. The main reason therefore is the degradation behavior of polymer on which the release profile is based on [11].

The intention of this study was to investigate the potential use of poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (PTA), a copolymeric polytartrate, as pulsatile release agent and to develop a biodegradable implant which is able to release vaccines or hormones in sharp boluses in two separate phases. PTA was selected due to its chemical architecture. In contrast to PLGA the polymer contains not only ester groups located in polymer backbone but also ester as well as ketal groups in polymer side chains [12]. Thus degradation can occur concomitant or successively in polymer main chain and polymer side chains which influences the degradation rate and in consequence the release behavior of PTA implants.

2. Materials and Methods

2.1 Materials

Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (Figure 1) was received from Aventis R&T, Frankfurt, Germany. PLGA, copolymer ratio 50:50 ($\overline{M}_w$ 41 kDa) and poly(L-lactic acid) (PLA, $\overline{M}_w$ 2000 Da) were purchased from Boehringer Ingelheim, Ingelheim, Germany. Magnesium stearate, tetrahydrofurane (THF), trifluoroacetic acid (TFA) and all further chemicals were purchased from Merck, Darmstadt, Germany. Buserelin acetate, M_w 1350 Da was obtained from Intervet International, Unterschleißheim, Germany.

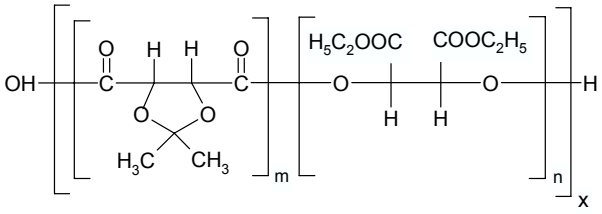

Figure 1 Poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate)

2.2 Molecular weight determination

The molecular weight was determined by size exclusion chromatography using two styragel columns (PSS guard column SDV, PSS SDV 100, 5 µm, PSS SDV linear M, 10 µm, 300 x 7.5 mm, Polymer Standard Service, Mainz, Germany). Degassed THF containing 0.1 % (v/ v) trifluoroacetic acid (TFA) was used as mobile phase at a flow rate of 1 ml/ min. A polymer aliquot (n=3) was dissolved in THF (20 mg/ ml) and filtrated before injection. The weight average molecular weight ($\overline{M}_w$) and the number average molecular weight ($\overline{M}_n$) were calculated relative to polystyrene standards (Polymer Standard Service, Mainz, Germany) using refractive index detection (ERC 7510, Tokyo, Japan) at 30 °C. Each sample was analyzed in duplicate and data were processed using ChromStar 4.1® software (SCPA, Stuhr, Germany).

2.3 Thermal analysis

Measurement of glass transition temperature (T_g) was performed using a differential scanning calorimeter (DSC 821, Mettler Toledo, Greifensee, CH). Two samples (~ 7 mg) of polymer were heated twice under nitrogen atmosphere. Thermograms covering a range from – 60 to 200 °C were recorded at a heating and cooling rate of 10 K/ min. Calibration of the system was performed using gallium and indium standards. The onset temperature which corresponds to the temperature at which the signal first deviates from baseline was used to describe the phase transition and was evaluated from the second heating run (STARe® software 6.0, Mettler Toledo, Greifensee, Switzerland).

2.4 Preparation of implants

Buserelin acetate and polymer powder (≤ 500 µm) were triturated in an agate mortar. Mixtures with different drug loadings (DL) were compressed at room temperature with appropriate pressure using two cylindrical flat-faced punches of 3 or 5 mm in diameter on a single punch excenter press (Korsch EK 0, Berlin, Germany). Implant weight was 14 or 40 mg, respectively.

The additive-containing implants were prepared in the same manner using a physical mixture of PTA and additional compound. The final drug polymer mixtures consisted 10 % buserelin by weight.

2.5 In vitro release

The weighed implants were placed into glass vials and 12 ml phosphate puffer (0.05 M, pH 7.4, containing 0.05 % benzalkonium chloride and 0.1% sodium azide) were added. The surfactant was used to minimize the adsorption of the peptide on the glass walls. Samples were incubated at 37 °C for 4 weeks without agitation. At defined time points 8 ml of the release medium were withdrawn and replaced by fresh buffer. Samples were analyzed for buserelin content using RP-HPLC. Separation was performed on a C18 column, 225 x 4 mm i.d. (Machery Nagel, Düren, Germany) at 42 °C. The mobile phase consisted of a mixture of buffer, pH 2.5 (triethylamine-containing phosphoric acid solution) and acetonitrile. The flow rate was 1 ml/ min. Detection was performed using a Waters 2470 programmable absorbance detector (Waters Corporation, Milford, USA) operating at 216 nm. Calibration curve was generated from known concentrations of buserelin acetate in mobile phase. The detection limit was 1 µg/ ml. The Millenium 32® chromatography manager, version 4.0 (Waters Corporation, Milford, USA) was used to analyze the data.

2.6 In vitro degradation

The in vitro degradation of pure PTA implants was studied under the same conditions as for in vitro release described above.

The weighed implants (n=4) were placed into weighted glass vials and 12 ml phosphate buffer (0.05 M, pH 7.4 containing 0.05 % benzalkonium chloride and 0.1 % sodium azide) were added. Samples were incubated at 37 °C without agitation for 4 weeks. After various time-periods four implants were removed, washed with demineralized water and lyophilized. The buffer solution was replaced after each sampling in order to prevent pH changes due to polymer degradation. The degree of polymer degradation was measured by SEC and DSC. Mass loss (ML) and water absorption (WA) were determined gravimetrically using the following equations:

(1) $ML\ (\%) = 100 \times (W_0 - W_d)/ W_0$

(2) $WA\ (\%) = 100 \times (W_w - W_d)/ W_d$

where W_0 is the implant weight determined initially, W_w is the wet weight of implant and W_d the weight of implant after lyophilization.

3. Results and discussion

The objective of this work was to investigate the potential use of poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) (Fig. 1) as pulsatile release agent. Implants were selected for their ease of manufacturing without the need to apply heat or organic solvents, which might reduce the activity of the drug. The size of the subcutaneous implant was selected in such a way that an administration through a wide-bore needle is possible.

An important factor in the design of a delivery system is the relative drug loading [13-15]. In order to evaluate the influence of drug loading on the release profile, the in vitro release from implants with various drug loadings (1, 5 and 10 % w/ w) was studied in buffer pH 7.4 at 37 °C.

Fig. 2 shows the daily and cumulative release profiles obtained from 3 mm implants at three different drug loadings. The multiphase release profile is characterized by an initial burst increasing concurrently with drug loading, releasing 5 to 25 % of drug within 24 hours. The initial burst can be attributed to the immediate release of buserelin entrapped at the implant surface. With decreasing drug loading the probability decreases that drug particles are situated on or near the surface thus the magnitude of initial burst is smaller. After 48 hours the initial burst was terminated and a secondary phase occurred, in which hardly any drug is released ("lag phase"). This can be explained by the fact that buserelin will be released mainly through water filled pores and channels. However, due to the chemical structure of PTA the polymer is initially lipophilic and only less pores formed by polymer densification during implant preparation are available for drug release. With increasing incubation time the lipophilic side chains, e.g. diethyl tartrate, were cleaved leading to an increase in polymer hydrophilicity and water absorption.

The fact that initially released buserelin creates also pores and channels for water absorption which facilitates hydrolysis, explains the small differences in the duration of lag phase. Depending on drug loading the lag phase lasted between 9 and 11 days until the second burst occurred. Within this third phase of release profile a great amount of drug is released as sharp peak and it was found that the extent of second burst ("booster dose") is related to drug loading and increases with decreasing drug loading. This can be explained with the lower initial burst and the resulting higher remaining drug content in implants of lower drug loading.

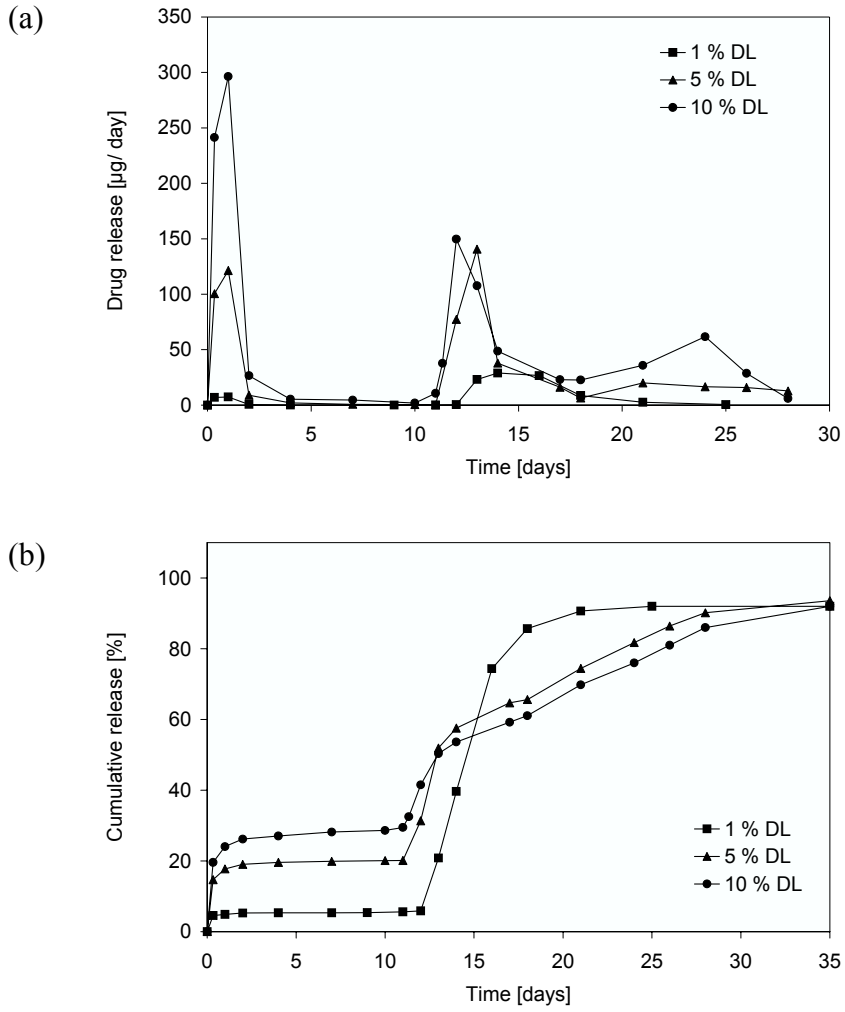

Figure 2 Differential (a) and cumulative (b) in vitro release of buserelin from PTA implants, 3mm in diameter in buffer at pH 7.4: effect of drug loading.

The linear relationship between initial and second burst shown in Fig. 3 and allows the adjustment of the booster dose. It was found that the second burst occurred after a dramatic change of implant shape. In parallel with an increase in water absorption the implant shape changed from a flat disc to a bloaded convex device. Within few days the polymer shell lacerated and the booster dose was released rapidly within 2-3 days. Afterwards, the remaining polymer mass released the remaining drug rather constantly at a slow release rate until the end of the study.

Another variable that is known to affect drug release is the implant size [16-18]. Therefore implants of 3 mm and 5 mm in diameter containing 10 % w/ w drug were prepared and their in vitro release behavior was evaluated (Fig. 4 a, b).

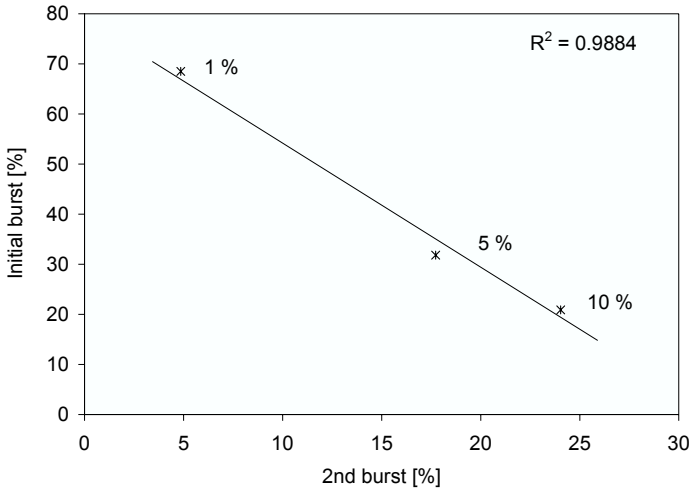

Figure 3 Cumulative in vitro release of buserelin from PTA implants, 3 mm in diameter in buffer (pH 7.4, 37 °C): Effect of drug loading on initial and second burst

A reduction of implant diameter resulted in an increase of initial burst (15 vs. 21 %) according to results published in literatur [19-21].

However, the overall release profile remained unchanged and a second burst was observed after a defined time intervall. It was found that the degree of the second burst as well as the amount of drug released thereafter increased with increasing implant size.

The drug release profile obtained from PTA implants is different to those reported for PLGA or PLA devices. The drug release from these polymer systems occurs in a triphasic manner, characterized by an initial burst, a lag phase where only small amounts of drug were released and a phase of increased drug release due to polymer erosion. During the polymer erosion phase a great amount of drug is released rather constantly over a longer time period. In contrast PTA implants released a great amount of drug within few days and the remaining drug is released at a lower release rate until the end of the study.

The observed second burst may be induced by polymer degradation. To verify this assumption the degradation of placebo PTA implants was evaluated under the same conditions as for the verum PTA samples. As generally known, the polymer molecular mass has to be reduced substantially to permit mass loss through solubilization [22-24].

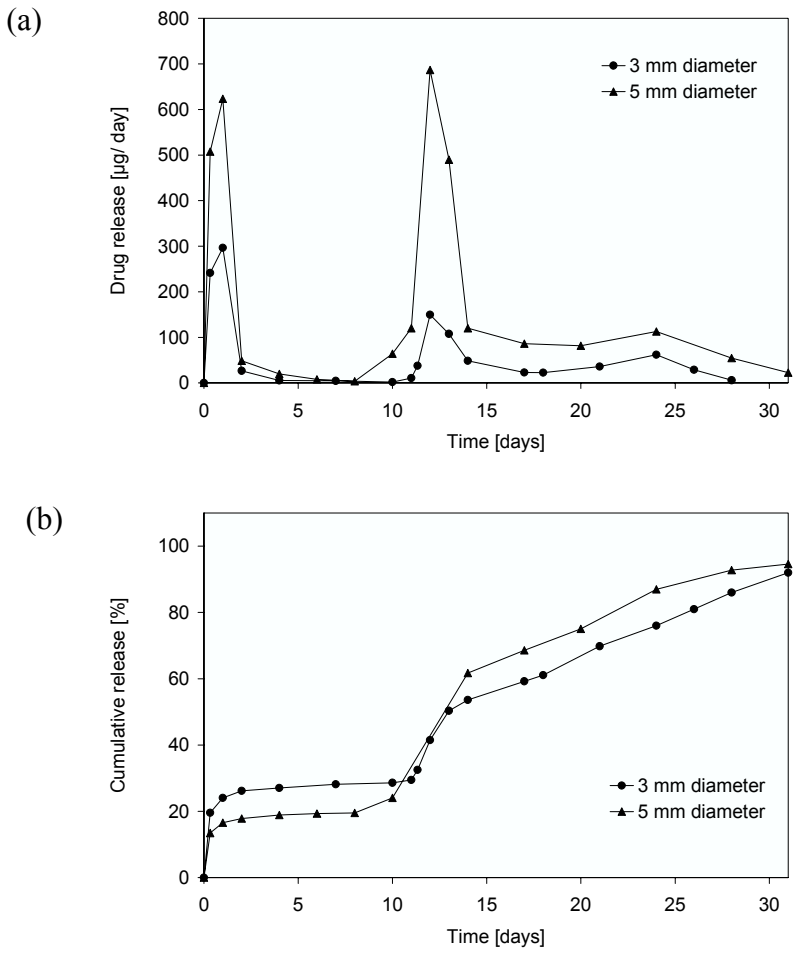

Figure 4 Differential (a) and cumulative (b) in vitro release of buserelin from PTA implants, 10 % drug loading, in buffer (pH 7.4, 37 °C): effect of implant diameter

From Fig. 5 it can be seen that the molecular weight decreased immediately after immersion of implants. However, neither mass loss nor water absorption were observed at the beginning. This can be explained by the fact that the rather lipophilic PTA contains hydrolysis labile bonds in both polymer backbone and polymer side chains [25]. During hydrolysis free acid functions as well as hydroxyl groups are formed which increase the hydrophilicity of the polymer and therefore facilitate water absorption. In consequence water absorption and mass loss commence after 7 days. After 2 weeks the molecular weight of remaining implant was dramatically decreased. This caused a further increase of implant permeability and hydrophilicity resulting in an accelerated mass loss. The implant mass decreased from 78 % at day 14 to 12 % at day 22 within one week. The remaining polymer mass was completely degraded until the end of the month.

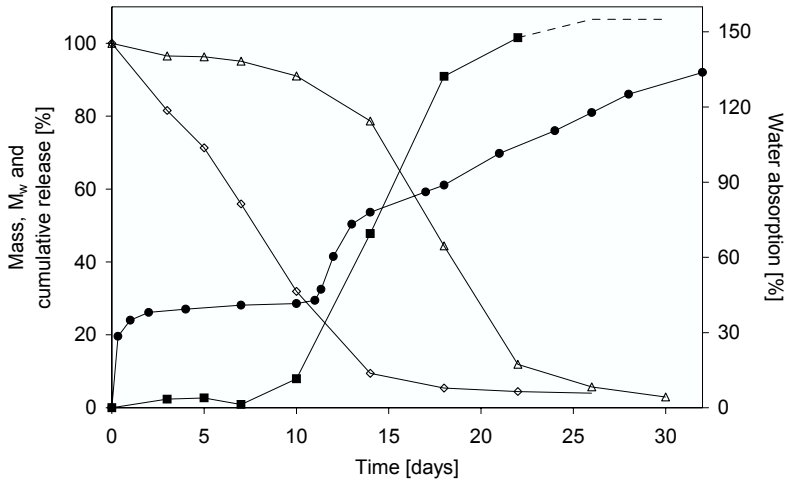

Figure 5 Mass loss (Δ), $\overline{M}_W$ decrease (◊), water absorption (■) and cumulative drug release (•) of PTA placebo implants (Δ, ■, ◊) and 10 % buserelin-containing implants (•).

The coincidence in degradation and release profils (Fig. 5) suggests that the second burst is mainly caused by polymer degradation. It was observed that both mass loss and release of booster dose occurred rapidly and in parallel with the elevation of implant shell. This can be explained by the fact that during degradation the molecular weight decreases whereas the polymer hydrophilicity raises due to the formation of hydroxylic and carboxylic end groups. As consequence water absorption increases and hence short polymer chains become flexible which causes the observed change in polymer shape. Based on the assumption that the implant borders are sintered due to fact that during preparation of implants polymer particles which were in direct contact to the compression tool were exposed to friction energy, the degradation products are enclosed within the polymer devices [26, 27]. With increasing degradation time the amount of small flexible chain fragments increases and the resulting inner pressure causes the elevation of implant shell.

The lag time before onset of implant bursting will depend on the polymer molecular weight, the copolymer ratio of applied PTA, the porosity and the size of the implant. Indeed, the induction period of PTA 3 mm was about 7 days, whereas it took 10 days for 5 mm implants (Thesis, Chapter 4). Furthermore, the physicochemical properties of drug will influence the degradation and finally the release profile. For hydrophobic drugs or compounds it is less likely that they dissolve in water and diffuse out of device due to their poor solubility. In consequence, less pores and channels will be

formed which could facilitate the absorption of water. Thus more time is needed to degrade the polymer and the second burst will occur at a later time point. In parallel with increasing molecular weight of PTA the time until implant bursting occurs will increase.

However, also admixing of polymers or excipients will affect the release profile. Therefore PLGA and PLA which differ in molecular size, hydrophilicity and sterical features were incorporated into PTA implants (10 % w/ w) and their effect on initial burst and booster dose was evaluated. Whereas PLGA is amorph and rather hydrophilic the latter is crystalline and more lipophilic than PLGA. Their glass transition and melting temperatures depend on molecular weight and polymer composition.

Based on preliminary test with aggregated lactose as model substance, which was released within 24 hours due to its excellent solubility, it was necessary to select a lipophilic water insoluble model substance. Thus magnesium stearate (Mg-sterate) which is commonly used as lubricant in a concentration range between 0.1 and 1 % w/ w was incorporated into the implant (10 % w/ w). After incubation of implant Mg-stearate located at the surface should be washed out to leave pores in the implant through which the drug can be released.

Buserelin, which is water-soluble will primarily be released through water filled pores and channels and less through a hydrophobic polymer matrix like PTA [28]. Leachable molecules, like Mg-stearate, will enhance drug release from the hydrophobic matrix. The results of in vitro release are summarized in Table 1.

The admixing of Mg-stearate resulted in an increase of initial burst from 24.0 to 39.5 % as expected and confirmed our assumption that a part of Mg-stearate is leached out and resulting pores increases drug release. In contrast the addition of PLGA leaded to a similar initial burst (22.8 vs. 24.0 %).

On the other side, the incorporation of the low molecular weight lipophilic and crystalline L-PLA resulted in an small increase of initial burst (24.0 vs. 29.2 %). Nevertheless, independent on incorporated materials the initial burst was followed by an extended lag phase until day 10.

The release of the booster dose was depending on incorporated material. It was found that incorporation of PLGA decreased the release rate during the second burst, most probably by swelling due to its high hydrophilicity compared to PTA.

Table 1 Drug release of different PTA implants containing 10 % w/ w buserelin

Implant	Drug release [µg]					
	Initial burst	Second burst				
	day 1	day 11	day 12	day 13	day 14	total
PTA	296	10.7	149.0	107.7	48.6	316.0
PTA/ Mg-stearate	643	9.9	22.8	32.8	41.7	107.2
PTA/ PLGA	335	10.6	61.2	106.2	84.8	262.8
PTA/ L-PLA	367	18.9	41.7	49.7	47.9	158.2

In contrast implants containing L-PLA or Mg-stearate did not show bursting of implant and no drug was released in a pulsatile manner. We assume that pores within the device which result either from leaching out of Mg-stearate or by diffusion of soluble degradation products of L-PLA prevent the accumulation of PTA degradation products that are responsible for the bursting of implant.

4. Conclusion

In this study poly(2,3-(1,4-diethyl tartrate)-co-2,3-isopropyliden tartrate) was identified as promising biodegradable polymer for pulsatile drug delivery.

The advantage of this polymer compared to other polyesters like PLGA is it's degradation behavior which results in bursting of implant after a definite time point. This allows the release of a great amount of drug within a short period of time after a definite lag phase where no drug is released and can be used for delivering drugs in a pulsatile manner.

The influence of the most important parameters like drug loading and implant size on drug release were investigated in this study. As expected, initial burst increased with increasing drug loading and decreasing implant size. All implants showed a second burst which was indirect proportional to initial burst. This allows the adjustment of the booster dose.

Moreover, the incorporation of polymers or leachable excipients like Mg-stearate can be used either to modify extent of secondary burst and the time at which implant bursting occurs or to inhibit this behaviour.

In the future other parameters have to be investigated such as molecular weight or copolymer ratio for achieving a bursting of implant at a predefined time point in a

range of days or weeks. Indeed, a challenge will be to achieve a uniform drug release during the bursting of implant. Nevertheless alternative depot formulations such as microparticles or in-situ implants could be prepared from PTA and their release characteristics could be investigated as alternative to implants.

References

[1] Cardamone M, Lofthouse SA, Lucas JC, Lee RP, O'Donoghue M, Brandon MR. In vitro testing of a pulsatile delivery system and its in vivo application for immunisation against tetanus toxoid. J. Control. Rel. 47 (1997) 205-219.

[2] Goepferich A. Bioerodible implants with programmable drug release. J. Control. Rel. 44 (1997) 271-281.

[3] Medlicott NJ, Tucker IG. Pulsatile release from subcutaneous implants. Adv. Drug Deliv. Rev. 38 (1999) 139-149.

[4] Carino G. Vaccine delivery. in: Mathiowitz E. (Ed.), Enzyclopedia of Controlled Drug Delivery. John Wiley, (1999), pp. 993-1006.

[5] Bowersock TL, Martin S. Vaccine delivery to animals. Adv. Drug Deliv. Rev. 38 (1999) 167-194.

[6] Rathbone MJ, Burns P, Ogle CR, Burggraaf S, Bunt CR. Controlled release drug delivery systems for estrous control of domesticated lifestock. in: Rathbone M, Gurny R (Eds.), Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam, (2000), pp. 201-228.

[7] Eldridge J, Staas J, Meulbroek J, McGhee T, Tice R, Gilley R. Biodegradable microspheres as a vaccine delivery system. Mol. Immunol. 28 (1991) 287-294.

[8] Ertl H, Varga I, Xiang Z, Kaiser K, Stephens L, Otvos L. Poly(D,L-lactide-co-glycolide) microspheres as carrier for peptide vaccines. Vaccine 14 (1996) 879-885.

[9] Hazrati A, Lewis D, Atkins T, Stohrer R, McPhillips C,Little J. Salmonella enteritidis vaccine utilizing biodegradable microspheres. Proc. Int. Symp. Control. Release Bioact. Mat., 1993, 101-102.

[10] O'Hagan D, Singh M, Gupta R. Poly(lactide-co-glycolide) microparticles for the development of single-dose controlled-release vaccines. Adv. Drug Deliv. Rev. 32 (1998) 225-246.

[11] Bowersock T, Martin S. Controlled release vaccines in veterinary medicine. in: Rathbone M, Gurny R (Eds.), Controlled Release Veterinary Drug Delivery. Elsevier Science, Amsterdam, (2000), pp. 269-309.

[12] Bengs H, Bayer U, Krone V, Lill N, Sandow J, Walch A. Polytartrate-a new biodegradable polymer. Proc. Int. Symp. ControI. Release Bioact. Mater. 23 (1996) 114-115.

[13] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21 (2000) 2475-2490.

[14] Lemmouchi Y, Schacht E, Lootens C. In vitro release of trypanocidal drugs from biodegradable implants based on poly(epsilone-caprolactone) and poly(d,l-lactide). J. Control. Rel. 55 (1998) 85-92.

[15] Ramchandani M, Robinson D. In vitro and in vivo release of ciprofloxacin from PLGA 50:50 implants. J. Control. Rel. 54 (1998) 167-175.

[16] Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 16 (1995) 305-311.

[17] Kader A, Jalil R. Formulation factors affecting drug release from poly(lactic acid) (PLA) microcapsule tablets.: Drug Dev. Ind. Pharm. 25 (1999) 141-151.

[18] Miyajima M, Koshika A, Okada J, Kusai A, Ikeda M. Factors influencing the diffusion-controlled release of papaverine from poly (l-lactic acid) matrix. J. Control. Rel. 56 (1998) 85-94.

[19] Wang F, Lee T, Wang C-H. PEG modulated release of etanidazole from implantable PLGA/PDLA discs. Biomaterials 23 (2002) 3555-3566.

[20] Lemmouchi Y, Schacht E. Preparation and in vitro evaluation of biodegradable poly(epsilone-caprolactone-co-d,l lactide)(X-Y) devices containing trypanocidal drugs. Journal of Controlled Release 45 (1997) 227-233.

[21] Okumu FW, Dao LN, Fielder PJ, Dybdal N, Brooks D, Sane S,Cleland JL. Sustained delivery of human growth hormone from a novel gel system: SABER®. Biomaterials 23 (2002) 4353-4358.

[22] Pitt CG. Non-microbial degradation of polyesters: mechanism and modifications. in: Vert M ,Feijen J, Albertsson A, Scott G, Chiellini E (Eds.), Biodegradable polymers and plastics. Royal Society of Chemistry, Cambridge, (1992), pp. 7-19.

[23] Shah S, Cha Y, Pitt C. Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery ? J. Control. Rel. 18 (1992) 261-270.

[24] Li S, Vert M. Biodegradable Polymers: Polyesters. in: Mathiowitz E. (Ed.), Encyclopedia of Controlled Drug Delivery. Wiley, John, New York, (1999), pp. 71-93.

[25] Ahlers M, Krone V, Walch A. Microparticles from biodegradable polymers. Adv. Mater. 4 (1992) 230-234.

[26] Guo HX, Heinämäki J, Yliruusi J. Characterization of particle deformation during compression measured by confocal laser scanning microscopy. Int. J. Pharm. 186 (1999) 99-108.

[27] Führer C. Interparticulate attraction mechanism. in: Alderborn G, Nystrom C. (Eds.), Pharmaceutical Powder Compaction Technology. Marcel Dekker, New York, (1996), pp. 1-15.

[28] Song CX, Labhasetwar V, Levy RJ. Controlled release of U-86983 from double-layer biodegradable matrices: effect of additives on release mechanism and kinetics. J. Control. Rel. 45 (1997) 177-192.

Chapter 6

In vitro and in vivo correlation of buserelin release from biodegradable implants using statistical moment analysis

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Abstract

Here we investigated the possibilty to develop different levels of correlation between in vitro drug release profiles and in vivo pharmacokinetic parameters for three Buserelin implant formulations. The in vitro and in vivo data were analyzed using model-independent and model-dependent methods. Since diffusion, dissolution and erosion effects influence drug release in most cases a simple kinetic model is unlikely to explain the overall in vivo release behavior. Thus the in vitro drug release curves were analyzed according to the theoretical models of Higuchi and Korsmeyer-Peppas. For the formulation with predominant diffusion controlled release level A IVIVC could be established (R^2= 0.986). Independent on drug release mechanism a level B correlation between the mean in vitro dissolution time (MDT) and mean in vivo residence time (MRT) was obtained with a correlation coefficient of 0.983. Finally, level C correlation were observed when single in vitro parameters, e.g. $T_{50\%}$ (time required to release 50% of drug in vitro) where compared with single in vivo parameters like AUC. This study suggests that a level B correlation could be achieved even when drug release occurs by a combination of diffusion and erosion processes. More sophisticated in vitro models mimicking drug release under in vivo conditions are clearly desirable for parenteral depot formulations.

1. Introduction

In the past decades several parenteral depot formulations based on biodegradable polymers such as microspheres and implants have become commercially available to improve the efficacy and prolong activity of several peptide and protein drugs [1-3]. Poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) are the most frequently used polymers for the preparation of these biodegradable delivery systems [4]. It is widely accepted that drug release from biodegradable delivery systems occurs by a combined mechanism of drug diffusion and polymer degradation or bioerosion [5, 6]. Factors, including polymer molecular weight, copolymer composition, crystallinity, and drug loading have a profound effect on drug release profiles [7-10]. As a result, drug release frequently does not follow a zero order kinetic which is desired from design perspective.

Different apparatus and methods have been developed to establish in vitro/ in vivo correlations (IVIVC) for biodegradable, parenteral dosage forms [11, 12]. However, only few examples can be found in literature where an in vitro drug method accurately predicts the in vivo release profile for parenteral biodegradable depot systems [13]. This demonstrates the difficulties in establishing IVIVC for this class of formulations due to the large number of parameters potentially affecting drug release in vivo and in vitro.

To establish IVIVC, at least three batches of the same drug substance should be available which differ in their in vivo as well in vitro performance [14, 15]. In general, the utilization of in vitro dissolution data for predicting in vivo performance requires a reliable method of data transformation. A comparison between in vitro and in vivo data is often not possible since the data describing in vivo release/ absorption profiles are not directly accessible. Various mathematical models and equations have been described in literature for conversion of measurable pharmacokinetic data to release/ absorption characteristics of the drug from the delivery systems for comparison with the in vitro release data [16, 17]. Depending on the method used to correlate the data, the USP established different levels of correlation, designated as A, B and C, in decreasing order of preferences and acceptability [15]. Level A IVIVC represents a point-to-point correlation between the in vivo absorption profile and the in vitro release profile. The in vivo absorption profile is calculated from plasma-concentration time curves using convolution or deconvolution methods. However, if a

level A correlation can not be established attempts should be made to correlate at level B. Level B correlations are based on statistical moment analysis and compare the mean in vitro dissolution time of the formulation with either the mean residence time in the body or the mean in vivo dissolution time of the formulation. Although a single parameter is compared in level B IVIVC, the method is known to be useful for extended release products.

Less informative is a level C IVIVC which may be useful for formulation development. This type of correlation represents a single point relationship and compares e.g. the time required for 50 % drug release in vitro with the area under the concentration time curve.

The intention of this investigation was the analysis of different levels of correlation for three different biodegradable implants by using model-dependent (Wagner-Nelson) and model-independent methods (statistical moment analysis). In order to obtain batches that differ in their in vitro performance implants which differ in drug loading were prepared by compression molding, then coated at different levels and characterized in vitro with respect to their release mechanism.

In addition, the relationship between release mechanism and quality of IVIVC level was assessed.

2. Materials and methods

2.1 Materials

Poly(D,L-lactide-co-glycolide) (PLGA), Type Resomer® RG 502H, lactide/glycolide ratio 50:50, molecular weight ($\overline{M}_w$) 17000 Da and poly(L-lactide) (L-PLA), Type Resomer® L104, $\overline{M}_w$ 2000 Da were purchased from Boehringer Ingelheim, Ingelheim, Germany. Profact Depot 2M®, batch C 109, was a gift from Aventis (Frankfurt, Germany). The sterile applicator contains two identical rods, each containing 3.3 mg buserelin acetate.

Buserelin acetate, M_w 1350 Da was obtained from Intervet International, Unterschleißheim, Germany. All further chemicals and solvents were purchased from Merck, Darmstadt, Germany.

2.2 Preparation of implants

The implants were obtained by compression molding of mixtures of polymer powder (≤ 500 µm) and buserelin acetate at various concentrations (10% to 30%). The mixtures with drug loadings (DL) of 10, 20 and 30 % w/w were compressed at 15 kN using two flat-faced punches of 3 mm in diameter on a single punch press (Korsch EK 0, Berlin, Germany) at room temperature in manual modus. The implant size was 3 mm x 1.6 mm and implant weight was approximately 14 mg. Implants for in vivo studies were placed in vials sealed under vacuum and gamma-irradiated (19.6 kGy) using a ^{60}Co source (Rüsch Sterilization Service, Kernen, Germany).

2.3 Coating of implants

PLGA implants with a diameter of 3 mm obtained by compression molding were coated with L-PLA solution (20 % w/v) in methylene chloride by spray coating technique. The process was controlled by increase in weight of the implants after evaporation of the solvent. The implants were dried for 24 hours at room temperature and the final coating thickness was calculated from mass increase. Implants for in vivo studies were sterilized by gamma irradiation as described above.

2.4 In vitro release study

The in vitro drug release studies were performed in 14 ml screw capped glass vials. The implants were placed into the vials and immersed with 12 ml phosphate puffer (0.05 M, pH 7.4) containing 0.05 % benzalkonium chloride and 0.1% sodium azide as antibacterial agents. Samples (n=6) were incubated at 37 °C for 4 weeks without agitation and were only shaken for 5 s at sampling time. At defined time points 8 ml of the release medium were withdrawn and replaced with fresh buffer. The removed medium was analyzed for amount of drug released by HPLC.

The release data were evaluated by model-dependent (curve fitting) and model-independent methods. For model-dependent analysis two theoretical models describing drug release from polymeric systems according to Higuchi [18] and Korsmeyer-Peppas were used [19, 20]. Higuchi describes drug release as a diffusion process based on Fick's law according to the equation:

$$Q = k_H \cdot t^{1/2} \qquad (1)$$

where Q is the amount of drug released in time t and k_H is the Higuchi dissolution constant. According to this model, a straight line is expected for the plot of Q versus the square root of time if drug release from the matrix is based on a diffusion mechanism.

The Korsmeyer-Peppas model takes into account that drug release mechanism often deviates from Fick's law and follows an anomalous behavior described by the following equation:

$$\frac{M_t}{M_\infty} = k \cdot t^n \qquad (2)$$

where M_t is the drug released at time t, M_∞ is the quantity of drug released at infinite time, k is the kinetic constant and n is the release exponent. The value of n is related to the geometrical shape of the delivery systems and determines the release mechanism. For cylindrical devices n is equal to 0.45 for diffusion-controlled release whereas n between 0.45 and 1.0 indicates an anomalous non-Fickian transport. Drug release from a porous system may lead to $n < 0.45$ due to the combination of diffusion through the matrix and partial diffusion through water-filled pores [20]. For evaluation of release data by the described models the portion of the release curve where $M_t / M_\infty < 0.6$ was used only as described in literature [18].

For statistical moment analysis the following model-independent in vitro parameter were determined: the mean dissolution time (MDT), percentage of drug released after 48 h (D_{48h}) and time for 50 % and 63.2 % drug release, respectively ($T_{50\%}$, $T_{63.2\%}$). The MDT was calculated according to the equation:

$$MDT = \frac{ABC_{in\,vitro}}{M_\infty} \qquad (3)$$

where ABC is the area between the release curve and its asymptote, calculated by the trapezoidal rule from time zero to the last measured time point, and M_∞ is the total amount of dissolved drug at this time point .

2.5 HPLC analysis

To determine drug loading and in vitro release, the drug concentrations were obtained by direct injection of samples into a HPLC system (Waters 2690, Waters Corporation,

Milford, USA). Samples were analyzed for buserelin content using RP-HPLC. Separation was performed on a C18 column (225 x 4 mm I.D., Machery Nagel, Düren, Germany) at 42 °C. The mobile phase consisted of a mixture of phosphoric acid, triethylamine buffer solution pH 2.5 and acetonitrile. The flow rate was 1 ml/ min. Detection was performed using a Waters 2470 programmable absorbance detector (Waters Corporation, Milford, USA) operating at 216 nm. Calibration curves were generated from known concentrations of buserelin acetate in mobile phase. The detection limit was 1 µg/ ml. The Millenium 32® chromatography manager, version 4.0 (Waters Corporation, Milford, USA) was used to analyze the data.

2.6 In vivo release study

The pharmacokinetic study reported here was approved by the public board for animal experiments. Mature male beagle dogs were used for all studies. They were housed in sin a lab dog colony at the Intervet animal farm according to the principles of laboratory animal care.

Dogs with a weight range of 10.6-15.4 kg were randomly divided into 3 groups (n = 4) and acclimated for at least 4 days prior the studies. Rectal temperature was measured before the experiment. Each dog was identified by ear tattoos and received one of the three formulations: uncoated implant (batch E), coated implant (batch F) both containing 4.2 mg buserelin or one of two identical rods of Profact depot 2M®, containing 3.3 mg buserelin (batch G). Using aseptic technique and local anaesthesia the implant was administered subcutaneously at the lateral part of the neck on the right body side of the dog using an applicator (Cerestar®, Intervet NL, Boxmeer).

At certain time intervals, from 4 hours to 35 days approximately 5 ml of blood samples were withdrawn from the Vena jugularis, Vena cephalica antebrachii or Vena saphena lateralis and collected in heparinized tubes. The plasma fraction of each blood sample was separated immediately by centrifugation and stored at –20 °C in labeled heparinized vials until analysis.

For determination of urinary excretion of buserelin dogs were placed in metabolic cages at definite time points for 12 hours. The urine samples were collected in plastic tubes and stored at –20 °C until assay. Plasma and urine concentrations of buserelin were determined by double-antibody radioimmunoassay [21](Aventis, Frankfurt, Germany).

2.7 Pharmacokinetic analysis

Pharmacokinetic parameters for buserelin following subcutaneous (s.c.) application in male beagle dogs were determined from the plasma and urine concentration-time data. The maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) were obtained directly from the individual plasma concentration-time data. The area of the first moment of the concentration-time curves (AUMC) and the area under the concentration-time curve from time zero to time t (AUC_{0-t}) were determined by the trapezoidal rule. The area from time t to infinity ($AUC_{0-\infty}$) was estimated according to the equation:

$$AUC_{0-\infty} = AUC_{0-t} + C_t / k_{el}$$

where C_t is the plasma concentration observed at time t, and k_{el} is the apparent elimination rate constant of buserelin obtained from the slope of the linear portion of the curve by least square regression analysis [22]. The mean residence time of the drug (MRT) and the absolute bioavailability (BV) of the formulations were calculated using the following equations:

$$MRT = \frac{AUMC}{AUC}$$

$$BV(\%) = \frac{AUC_{sc}}{AUC_{iv}} \times \frac{dose_{iv}}{dose_{sc}} \times 100$$

where AUC_{sc} and AUC_{iv} are the area under the curve after s.c. and i.v. administration, respectively. $Dose_{iv}$ and $dose_{sc}$ denotes the amount of drug administered intravenously and subcutaneously, respectively.

Utilizing the i.v. kinetic parameters obtained in a preliminary study [23], the in vivo percent of drug absorbed after s.c. administration at each time period was estimated by the Wagner-Nelson method [17]. Drug amounts are assessed from rate constants of the i.v. two compartment model for each dog and the cumulative area under the curve for each dog following s.c. administration of the buserelin implants. Calculations were carried out with pharmacokinetic software, Kinetica 2000, version 3.0 (Innaphase, Philadelphia, USA). In the compartmental fit the weighting factor was $1/C_p^2$ (C_p is the experimental plasma concentration).

2.8 Statistical evaluation

Results are expressed as mean ± SD of four dogs per group. Data analysis of the pharmacokinetic parameters was performed by Student's t-test. Differences between two related parameters were considered statistically significant for p values of or less than 0.05.

2.9 In vitro-in vivo correlation

Three levels, A, B, and C, of in vitro-in vivo correlation (USP 26) were studied.
For Level C IVIVC the single release parameters $T_{50\%}$, $T_{63.2\%}$, and D_{48h} were compared with the single in vivo parameters $AUC_{0\text{-}24h,}$ MRT and C_{max}.
In order to establish Level B IVIVC the mean in vitro dissolution time was compared with the mean in vivo residence time. For Level A IVIVC the fraction absorbed in vivo, generated by the Wagner-Nelson method, was plotted versus the fraction released in vitro at the same time. As alternative method to demonstrate correlation the time in vivo for absorption versus the time in vitro for release of the same amount of drug was plotted [24]. If there is level A IVIVC, the curves are directly superimposable or can be made superimposable by using the time scaling factor and/ or the constant offset value [25]:

$$t_{in\ vivo} = a + b \cdot t_{in\ vitro}$$

where $t_{in\ vivo}$, $t_{in\ vitro}$ are times in vivo and in vitro, respectively, *a* is the lag time in vivo and *b* is the time scale factor. The time scale factor takes into account that in vitro release and in vivo absorption do not always follow the same time scales.

3. Results and discussion

3.1 In vitro release study

For a drug incorporated in PLGA-based matrices, several possible mechanisms may be involved in the release processes: drug diffusion from implants, matrix erosion resulting from degradation and dissolution of PLGA polymer and the combination of the above two rate processes [26-28]. Furthermore it is known that drug loading and drug particle size distribution determine the geometry and the topology of pores and channels which determine drug diffusion from the hydrophobic matrix [29]. Drug

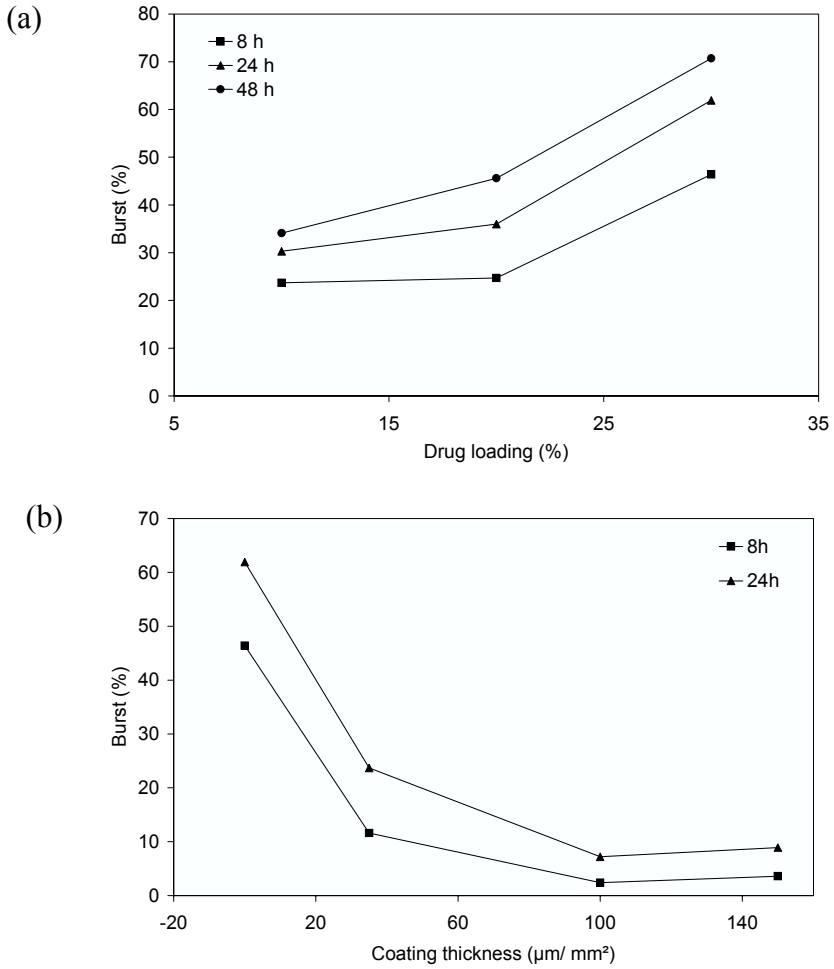

Figure 1 In vitro drug release from PLGA disc-shaped implants: (a) Influence of drug loading on burst effect and (b) Effect of coating on initial release as function of coating thickness. Data are shown as an average ± S.D. (n = 6).

loading affects drug release by changing the porosity of the matrix because channels are formed as solid drug is dissolved and released. Such percolation effects in matrix-type controlled delivery systems were extensively studied by Leuenberger et al. [30, 31].

The effect of drug loading on initial drug release of buserelin from PLGA implants is shown in Fig. 1a. The drug burst increased significantly with increasing drug loading which suggests that buserelin was directly dissolved after contact with buffer and diffused into the aqueous medium as expected.

In order to reduce the drug burst and therewith to retain drug within the matrix implants containing 4.2 mg buserelin were coated with low molecular weight L-PLA.

The coating had a thickness of 35, 100 or 150 µg/ mm^2 calculated from mass increase (Table 1). The effect of three coating levels on drug release under in vitro conditions is shown in Fig. 1b. The initial burst decreased with increasing coating thickness. Already at a coating of 35 µg/ mm² a 57 % reduction of the initial release from 2.4 mg/d to 1.03 mg/d at peak maximum was observed. By increasing the coating thickness from 35 to 100 µg/ mm² the reduction of the drug burst was further improved by 87 % (from 2.4 mg/d to 0.32 mg/d release). Further coating to a thickness of 150 µg/ mm² did not affect drug release any further. However, in contrast to results reported in literature [32, 33] a lag-phase of drug release caused by PLA coating during the first week was not found.

This could be explained by the assumption that after spray-coating of implants residues of solvent are still in the coating layer resulting in a film of increased permeability. The fact that coating and drying was performed below the glass transition temperature of the coating polymer contributes to the permeability of film. Thus the drug from coated implants is released by diffusion through pores and the polymer coating film. The remaining minimal burst effect has a biological component and is beneficial because a higher initial release ensures a prompt effect, which can be subsequently maintained for a prolonged period by a slower but continuous release of buserelin.

Table 1 Kinetic assessment of release data for different buserelin implants

Batch	Drug loading (mg)	Coating (µg/mm²)	Higuchi		Korsmeyer-Peppas[1]	
			Slope	R²	slope	R²
A	4.2	0	30.91	0.978	0.343	0.982
B	4.2	35	26.46	0.990	0.305	0.993
C	4.2	100	14.28	0.991	0.172	0.990
D	4.2	150	13.07	0.989	0.228	0.991
E*	4.2	0	31.28	0.996	0.384	0.997
F*	4.2	150	16.75	0.992	0.248	0.999
G**	3.3	0	19.39	0.995	0.233	0.998

[1] release exponent $n = 0.45$
* gamma-irradiated with 19.6 kGy
** one of the two rods of Profact Depot 2M®

In order to determine if the release mechanism of coated implants is governed by Fickian diffusion, the results were fitted to both the Higuchi and the Korsemeyer-Peppas model (n=0.45). According to these models, a straight line is expected foreach plot if drug release from the matrix is based on a diffusion mechanism. In both cases good correlation coefficients were obtained suggesting that drug release from these implants was consistent with a Fickian diffusion mechanism (Table 1).

For in vivo studies a second set of implants (see Table 1) was prepared, sterilized and characterized in vitro. The gamma-irradiation of implants causes a slight decrease in polymer molecular weight from 15.9 kDa to 14.3 kDa [34].

The in vitro drug release of these implants was investigated in a comparative study using a commercial available implant (Table 1, batch G). The release profiles are shown in Fig. 2. The uncoated implant (batch E) showed a biphasic release pattern, with an high initial burst followed by a slower release phase. In contrast, implants of identical drug loading coated with 150 µg/mm² L-PLA (batch F) released the drug rather constantly. Since the degradation time of coating and matrix polymer was similar to the drug release time no lag phase was observed and a daily release of 40 µg buserelin was achieved over a period of 4 weeks. A longer drug release was observed from batch G implants. During the first 2 weeks drug release from these rods was faster than from coated implants and more than 69 % of drug was released in

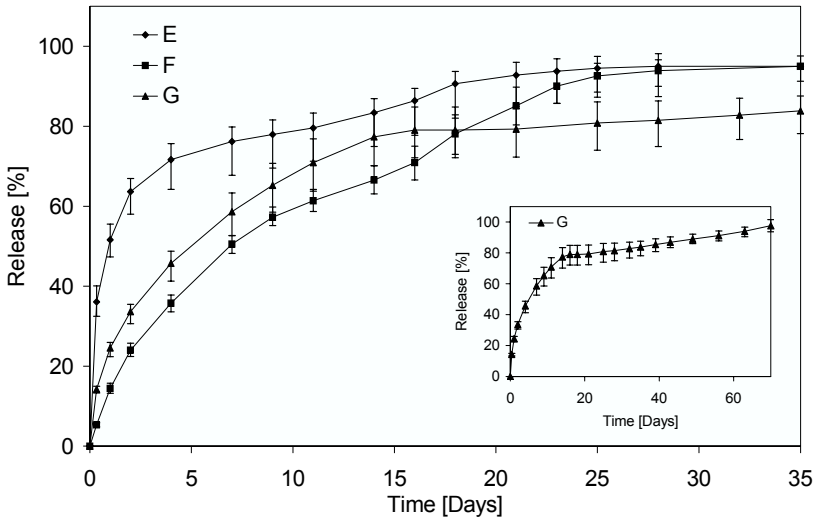

Figure 2 In vitro cumulative release profiles for buserelin batches used in the pharmacokinetic study up to 35 days. The insert shows the release profile of batch G implants for 70 days. Data are shown as an average ± S.D. (n = 4-6).

vitro until this time point. However, thereafter drug release rate decreased rapidly and only small amounts of drug (~ 10 µg/day) were released constantly over 70 days (Fig. 2, insert). The result is related to the longer degradation time of polymer where batch G implants are based on. The fitting of these data to the Korsmeyer-Peppas model demonstrated that drug release within the first 2 weeks occurs mainly by diffusion (n = 0.45, R^2 = 0.998). Thereafter a combination of diffusion and erosion processes dominated until the end of the study since the value of *n* was lower than 0.45 (n = 0.22, R^2 = 0.997).

3.2 In vivo release study

Based on the in vitro results for developing IVIVC three formulations were selected for in vivo evaluation: uncoated implant (E) as the fast-releasing implant, which was thought to give the highest initial plasma levels, coated implant (F) which was expected to lead to smaller initial plasma levels and thus to a prolonged release phase, and Profact Depot 2M® (G) which was thought to give small plasma levels after 2 weeks as observed in vitro.

Fig. 3a shows the in vivo plasma buserelin concentration-time profiles after i.v. injection of buserelin solution [23]. The rapid decrease in buserelin concentration after i.v. administration reflects the fast disposition and elimination of the drug. The plasma concentration-time profiles can be described by a two compartment model according to the equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is the plasma concentration of buserelin at time t; A and B are empirical constants; and α and β are hybrid rate constants.

The rate constants α and β were derived from the slopes of the decline in plasma concentration during the initial distribution and the terminal elimination phase. From the estimated slopes the half-lifes of α and β phase were calculated as $t_{1/2\alpha} = 0.693/\alpha$ and $t_{1/2\beta} = 0.693/\beta$.

The elimination rate constants of α and β phase were found to be 0.162 and 0.012 min^{-1}, respectively and the half-lifes of α and β phase were calculated as $t_{1/2\alpha} = 0.693/\alpha$ and $t_{1/2\beta} = 0.693/\beta$. The obtained half-lifes were 4.3 min (α-phase) and 56.4 min (β phase). The half-life of buserelin obtained from terminal phase is in good

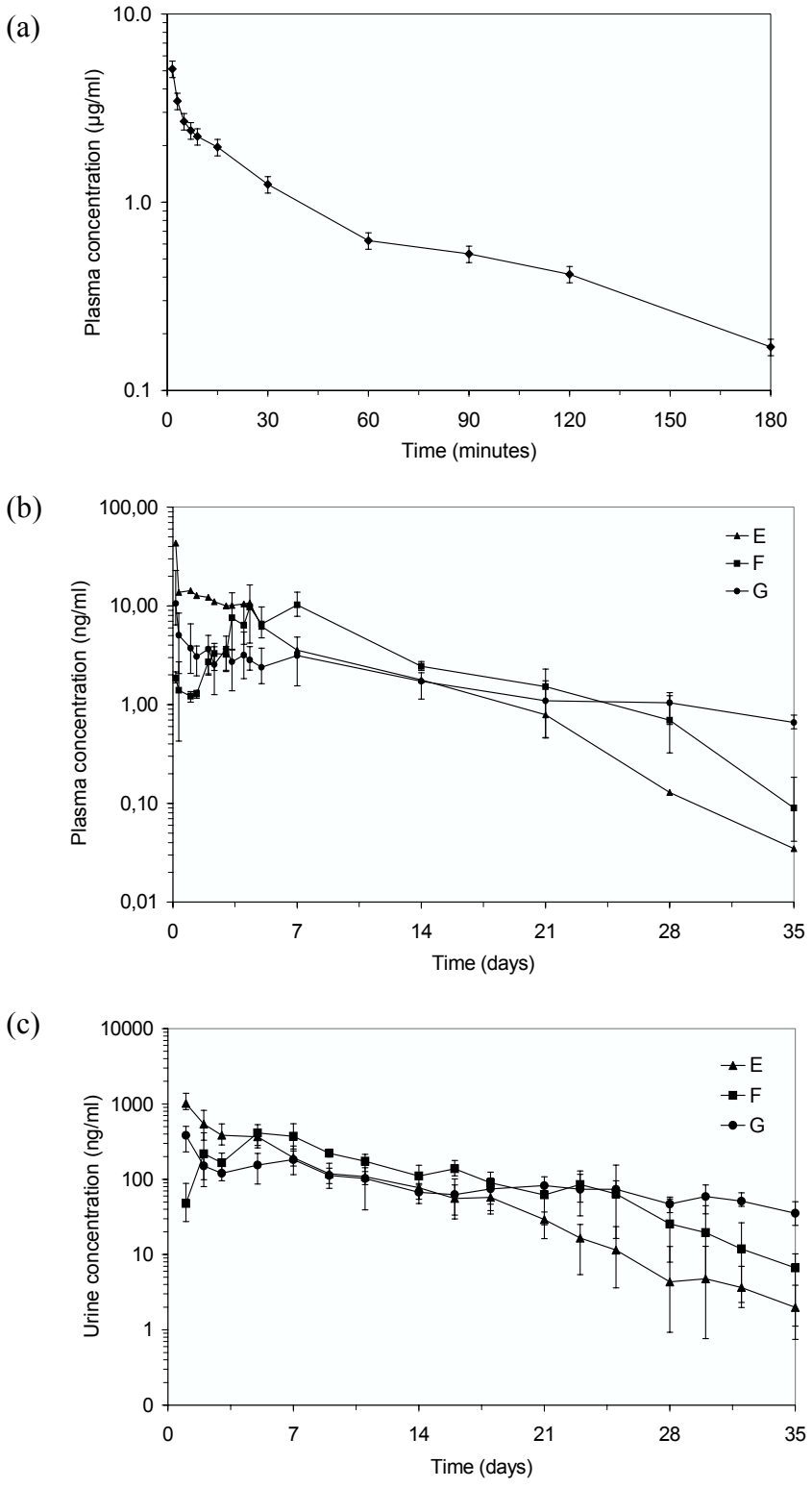

Figure 3 In vivo release profiles in dogs: (a) Plot of plasma concentration of buserelin vs. time after i.v. administration, (b) Plot of plasma buserelin concentration vs. time and (c) Plot of urine buserelin concentration vs. time after s.c. administration of formulation A, B and C. Data are shown as an average ± S.D. (n = 3-4).

Table 2 Pharmacokinetic parameters following single i.v. administration of buserelin (5mg/dog)

Parameter	Mean	S.D.
α (min^{-1})	0.162	0.032
β (min^{-1})	0.012	0.001
$t_{1/2\alpha}$ (min)	4.3	0.6
$t_{1/2\beta}$ (min)	56.4	0.98
Cl (ml/kg/min)	1.7	0.10
Vc (ml/kg)	50.1	2.4
AUC (μg·ml/min)	139.6	12.4
Vz (ml/kg)	135.9	7.9
Vss (ml/kg)	118.2	5.5
MRT (min)	70.7	2.8

Cl total clearaence
Vc volume in the central compartment
Vz apparent volume of distribution during the terminal phase
Vss apparent volume of distribution at steady state

agreement with reported values of 25-80 min [35, 36]. The short half-life of buserelin explains the rapid decline of plasma concentrations after i.v. administration. The pharmacokinetic parameters are summarized in Table 2.

The mean plasma concentration-time profiles of buserelin after s.c. administration of formulations A, B and C are shown in Fig. 3b.

Since the daily release from tested formulations is low, the plasma concentrations of buserelin are very low compared to urine samples where due to the renal excretion higher concentrations are reached. Thus urine samples were taken additionally and the mean urine profiles of investigated formulations are plotted in Fig. 3c. Table 3 reports the mean values of pharmacokinetic parameters obtained when the three studied formulations were administered subcutaneously. The results were calculated from plasma and urine data by non-compartmental analysis [22].

After administration of formulation E, containing 4.2 mg drug per implant, high initial plasma (C_{max} 43.1 ± 5.6 ng/ ml at 4 hrs) and urine levels (C_{max} 1018 ± 250 ng/ ml at 24 hrs) were observed. Thereafter the plasma concentration dropped rather linear below 1 ng/ ml. By comparison, formulation F of equivalent dose produced only

Table 3 Mean (± S.D.) pharmacokinetic parameters following s.c. administration of buserelin in dogs (n = 4)

Formulation	C_{max}[a] (ng/ml)	T_{max}[b] (h)	AUC_{0-1}[c] (ng/ml·day)	AUC_{0-4}[d] (ng/ml·day)	$AUC_{0-\infty}$[e] (ng/ml·day)	MRT[f] (d)	BV[g] (%)
E	43.1 ± 5.6*	4 ± 0*	17.6 ± 4.7*	51.5 ± 11.4*	101.9 ± 12.0*	3.8 ± 1.4*	84.2 ± 17.3*
	1018 ± 250**	24 ± 0**	n.d.**	n.d.**	4262 ± 752**	6.6 ± 0.6**	n.d.**
F	13.1 ± 3.7*	146.4 ± 37*	1.3 ± 0.6*	17.7 ± 12.2*	106.2 ± 14.5*	9.9 ± 1.5*	92.8 ± 17.9*
	508 ± 55**	136.8 ± 27**	n.d.**	n.d.**	4370 ± 574**	11.0 ± 1.0**	n.d.**
G	14.1 ± 8.3*	4 ± 0*	8.2 ± 3.0*	20.6 ± 5.3*	79.9 ± 8.5*	15.6 ±3.7*	73.1 ± 14.3*
	405 ± 103**	24 ± 0**	n.d.**	n.d.**	3303 ± 197**	18.4 ± 3.7**	n.d.**

* obtained from plasma data

** obtained from urine data

a maximum plasma/ urine concentration

b time to achieve maximum concentration

c area under the plasma/ urine concentration-time curve from zero time to 24 hours

d area under the plasma/ urine concentration-time curve from zero time to 4 days

e area under the plasma/ urine concentration-time curve from zero time to infinity

f Mean residence time

g bioavailability

n.d. not determined

Table 4 ANOVA-test for pharmacokinetic parameters determined from plasma data of different buserelin implants

Parameter	E vs. F	F vs. G
C_{max}	P< 0.05	n.s.
T_{max}	P< 0.05	P< 0.05
MRT	P< 0.05	P< 0.05
AUC_{0-1}	P< 0.01	P< 0.05
AUC_{0-4}	P< 0.05	n.s.
$AUC_{0-\infty}$	n.s.	P< 0.05

n.s. no statistically significant differences

P-value indicated the comparison of values of each groups obtained by Student's *t*-test

negligible buserelin plasma and urine levels (< 1 ng/ ml) during the first days increasing with time. The peak plasma (C_{max} 13.1 ± 3.7 ng/ ml) and urine concentration (C_{max} 508.7 ± 55 ng/ ml) were smaller than those obtained with formulation E. A statistically significant difference was found between formulation E and F in both time and magnitude of the generated peak ($p < 0.05$). No statistical difference was found between the two formulations in AUC_{0-35d}, indicating a comparable extent of absorption (Table 4).

Formulation G, containing only 3.3 mg drug, released buserelin rather constantly after an initial burst. The peak plasma (C_{max} 14.1 ± 8.3 ng/ ml) and urine concentration (C_{max} 405 ± 103 ng/ ml) were similar to those obtained with formulation F. There were no statistically significant differences between the absolute plasma and urine concentrations until day 25 (Table 4). Thereafter the mean concentration level of formulation G was higher than that obtained from formulation F. The MRT of formulation G was significantly longer compared to formulation F (15.6 vs 9.9 days, $p < 0.05$) (Table 4). However, it should be noted that for formulation G the value obtained for MRT is underestimated due to the fact that this formulation is based on a polymer which degradation time is longer than the duration of the in vivo study (see Fig. 2, insert). In order to estimate the amount of drug released from the tested formulations the absolute bioavailability (BV) was calculated from the AUC of i.v. injection and the administered dose. The absolute bioavailabilities were 84 ± 17 %, 93

± 18 % and 73 ± 14 % for formulations E, F and G. The results indicate that all incorporated drug was released completely from formulation E and F whereas in formulation G a small amount of drug was still available in the implant at the end of the study.

3.3 In vitro in vivo correlation

Level C in vitro-in vivo correlation was investigated for $T_{63.2\%}$ versus MRT, C_{max} in vivo versus the percentage of drug released in vitro after 48 hours (D_{48h}) and between $T_{50\%}$ and $AUC_{0\text{-}1d}$. A good correlation was observed between these parameters and correlation coefficients varied from 0.977 to 0.999 (Table 5). No correlation was found between $T_{50\%}$ and C_{max} in vivo.

Since level C correlation represents a single point comparison and does not reflect the complete shape of the plasma level profile this type of correlation is not predictable of actual in vivo performance and is generally only useful as reference in formulation development or as a product quality control reference procedure. Level B IVIVC was investigated for MDT in vitro versus MRT in vivo and a good correlation was obtained ($R^2 = 0.98$).

Table 5 Correlation coefficients between in vitro dissolution and in vivo pharmacokinetic parameters

In vitro parameter	In vivo parameter	R^2	slope	intercept
MDT [a]	MRT [b]	0.983	1.0224	-1.7477
$T_{63.2\%}$ [c]	MRT	0.9863	0.9318	-1.888
$T_{50\%}$ [d]	$AUC_{0\text{-}24h}$ [e]	0.9993	-2.0299	19.914
D_{48h} [f]	C_{max} [g]	0.9774	0.8354	-8.3737

[a] Mean dissolution time

[b] Mean residence time

[c] Time required to dissolve 63.2% of drug

[d] Time required to dissolve 50% of drug

[e] Area under the serum concentration-time curve from zero time to 48 hrs

[f] Percentage of drug dissolved after 48 hrs

[g] Maximum serum concentration

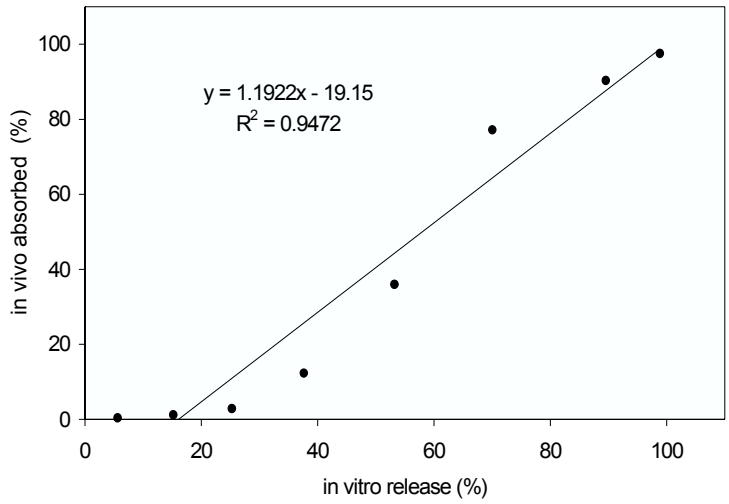

Figure 4 Plot of percentage of dose absorbed in vivo versus percent released in vitro

However, a level A IVIVC is considered to be the most informative and is thus recommended for regulatory purposes. Therefore, the amounts of drug absorbed after s.c. administration of formulation F which was of interest for further development, was calculated by the Wagner Nelson method using the linear trapezoidal rule.

For testing a level A IVIVC two methods were used: (1) the percentage of drug absorbed up to time t was plotted versus the amount of drug released in vitro (Fig. 4) and (2) the time in vivo for 0-10-20-30-40-50-60-70-80-90 % absorbed was plotted versus the time for in vitro release of the same amounts of drug (Levy plot) (Fig. 5).

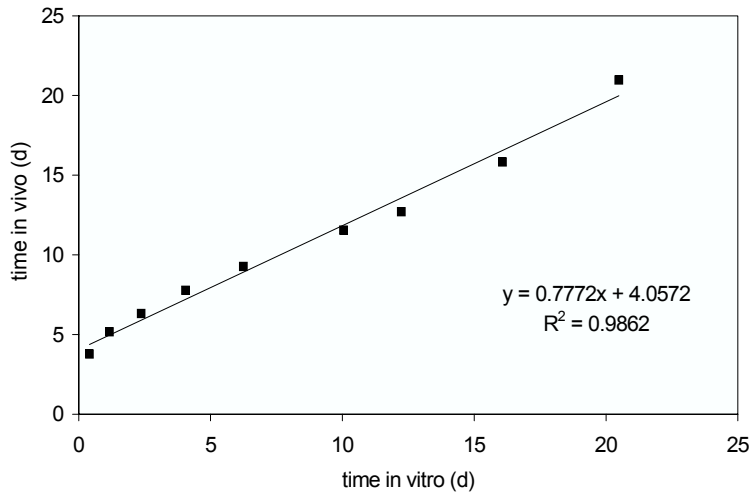

Figure 5 Levy plot obtained from times for 0-90 % buserelin released in vitro and absorbed in vivo

Visual inspection of the plots suggests a good correlation of the release profiles.
However, the best correlation coefficient was obtained when using the Levy plot. The relationship obtained from the Levy plot indicates that the curves are not superimposable due to the different slope and an intercept different from zero. From the intercept the lag time in vivo was estimate to be 4.05 d.

4. Conclusion

The release of buserelin from three different biodegradable implants was determined in vitro and compared to the pharmacokinetic profile using model-independent and model-dependent methods.
Level A IVIVC ($R^2 = 0.986$) was found for batch E implants. Drug release from these implants could be described by the Higuchi model over the entire release period of 4 weeks. For implants where drug release occurs by a combination of diffusion, dissolution and erosion processes a level B IVIVC could be established. Due to the fact that the development of a suitable release test for biodegradable delivery systems is still a major problem alternative approaches for prediction of in vivo performance are needed. In this study we could demonstrate that a comparison of MDT in vitro versus the MRT in vivo yielded in a level B IVIVC although no sophisticated release model such as flow-through cell or dialysis bags were used. Moreover, it could be shown that the in vitro parameter $T_{63.2\%}$ can be used instead of MDT in order to establish this level of correlation. This means that for formulation development or as quality control method for clinical samples an in vitro test may be sufficient which monitors drug release until this time. By using model-independent methods also a good correlation between in vitro and in vivo parameters was obtained.

References

[1] Morlock M, Koll H, Winter G, Kissel T. Microencapsulation of rh-erythropoietin, using biodegradable poly(-lactide-co-glycolide): protein stability and the effects of stabilizing excipients. Eur. J. Pharm. Biopharm. 43 (1997) 29-36.

[2] Cleland JL, Duenas E, Daugherty A, Marian M, Yang J, Wilson M, Celniker AC, Shahzamani A, Quarmby V, Chu H. Recombinant human growth hormone poly(lactic-co-glycolic acid) (PLGA) microspheres provide a long lasting effect. J. Control. Rel. 49 (1997) 193-205.

[3] Rothen-Weinhold A, Besseghir K, De Zelicourt Y, Gurny R. Development and evaluation in vivo of a long-term delivery system for vapreotide, a somatostatin analogue. J. Control. Rel. 1-2 (1998) 205-213.

[4] Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Deliv. Rev. 28 (1997) 5-27.

[5] Furr BJA, Hutchinson FG. A biodegradable delivery system for peptides: preclinical experience with the gonadotrophine-releasing hormone agonist Zoladex. J. Control. Rel. 21 (1992) 117-128.

[6] Shah S, Cha Y, Pitt C. Poly(glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery ? J. Control. Rel. 18 (1992) 261-270.

[7] Hurrell S, Cameron RE. The effect of initial polymer morphology on the degradation and drug release from polyglycolide. Biomaterials 23 (2002) 2401-2409.

[8] Kader A, Jalil R. Formulation factors affecting drug release from poly(lactic acid) (PLA) microcapsule tablets.: Drug Dev. Ind. Pharm. 25 (1999) 141-151.

[9] Park TG. Degradation of poly(D,L-lactic acid) microspheres: effect of molecular weight. J. Control. Rel. 30 (1994) 161-173.

[10] Rothen-Weinhold A, Besseghir K, Gurny R. Analysis of the influence of polymer characteristics and core loading on the in vivo release of a somatostatin analogue. Eur. J. Pharm. Sci. 5 (1997) 303-313.

[11] Shah V, Siewert M, Dressman J, Moeller H, Brown CK. Dissolution/ In vitro Release Testing of Special Dosage Forms. Dissolution Technologies 9 (2002) 1-5.

[12] Dash AK, Haney PW, Garavalia MJ. Development of an in vitro dissolution method using microdialysis sampling technique for implantable drug delivery systems. J. Pharm. Sci. 88 (1999) 1036-40.

[13] Woo BH, Kostanski W, Gebrekidan S, Dani BA, Thanoo BC, DeLuca PP. Preparation, characterization and in vivo evaluation of 120-day poly(D,L-lactide) leuprolide microspheres. J. Control. Rel. 75 (2001) 307-315.

[14] Uppoor VRS. Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations. J. Control. Rel. 72 (2001) 127-132.

[15] USP. USP 26/ NF 21. Pharmacopial Convention. Inc., Rockville, (2003).

[16] Langenbucher F. Linearization of dissolution rate curves by the Weibull distribution. J. Pharm. Pharmacol. 24 (1972) 979-981.

[17] Wagner JG, Nelson E. Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drug. J. Pharm. Sci. 53 (1964) 1392-1403.

[18] Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 52 (1963) 1145-1149.

[19] Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 15 (1983) 25-35.

[20] Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm. Acta Helv. 60 (1985) 110-111.

[21] Holland F, Fishman L, Luder A, Costigan D, Jenner M, Wielgosz G, Fazekas A. Subcutaneous and intranasal D-Ser(tBut)^{9}EA^{10} LHRH (Buserelin) in the management of precocious puperty. in: Labrie F, Belanger A, Dupont A (Eds.), LHRH and its analogues. Elsevier Science Publishers B.V., (1984), pp. 407-421.

[22] Gibaldi M, Perrier D. Pharmacokinetics, 2nd. edition, Marcel Dekker, New York, 1982.

[23] Sandow J, Jerabek-Sandow G, Krauss B, Schmidt-Gollwitzer M. Pharmacokinetics and metabolism of LHRH agonists, clinical aspects. in: Labrie F, Belanger A, Dupont A (Eds.), LHRH and its analogues. Elsevier Science Publishers B.V., (1984), pp. 123-137.

[24] Mojaverian P, Rosen J, Vadino WA, Liebowitz S, Radwanski E. In-vivo/in-vitro correlation of four extended release formulations of pseudoephedrine sulfate. J. Pharm. Biomed. Anal. 15 (1997) 439-445.

[25] Brockmeier D, Voegele D, von Hattingberg HM. In vitro-in vivo correlation, a time scaling problem? Arzneim. Forsch. 33 (1983) 598-601.

[26] Bodmer D, Kissel T, Traechslin E. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J. Control. Rel. 21 (1992) 129-138.

[27] Chiu LK, Chiu WJ, Cheng YL. Effects of polymer degradation on drug release-a mechanistic study of morphology and transport properties in 50:50 poly(dl-lactide-co-glycolide). Int. J. Pharm. 126 (1995) 169-178.

[28] Narasimhan B, Peppas NA. Molecular analysis of drug delivery systems controlled by dissolution of the polymer carrier. J. Pharm. Sci. 86 (1997) 297-304.

[29] Holland SJ, Tighe BJ, Goulg PL. Polymers for biodegradable medical devices. 1. The potential of polyesters as controlled macromolecular release systems. J. Control. Rel. 4 (1986) 155-180.

[30] Leuenberger H, Holman L, Usteri M, Winzap S. Percolation theory, fractal geometry and dosage form. Acta Helv. 64 (1989) 34-39.

[31] Bonny JD, Leuenberger H. Matrix type controlled release systems. I. Effect of percolation on drug dissolution kinetics. Pharm. Acta Helv. 66 (1991) 160-164.

[32] Baro M, Sanchez E, Delgado A, Perera A, Evora C. In vitro-in vivo characterization of gentamicine bone implants. J. Control. Rel. 83 (2002) 353-364.

[33] Vandamme TF, Ngombo Mukendi JF. Controlled release of levamisole from poly(epsilon-caprolactone) matrices: III. Effect of molecular weight and polymer coating on drug release. Int. J. Pharm. 145 (1996) 77-86.

[34] Volland C, Wolff M, Kissel T. The influence of terminal gamma-sterilization on captopril containing poly(D,L-lactide-co-glycolide) microspheres. J. Control. Rel. 31 (1994) 293-305.

[35] Sandow J, Jerabek-Sandow G, Krauss B, von Rechenberg W. Pharmacokinetic and antigenicity studies with LHRH analogues, in: B. Runnebaum, T. Rabe, L. Kiesel (Eds.),

Future aspects in contraception, part 2, female contraception, MTP press Ltd., Boston, 1985, pp. 129-147.

[36] Sandow J, Fraser HM, Seidel H, Krauss B, Jerabek-Sandow G, von Rechenberg W. Buserelin: pharmacokinetics, metabolism and mechanisms of action. Br. J. Clin. Pract. 41 (1987) 6-13.

Conclusion

Summary

This thesis deals with the degradation and release behavior of aliphatic polyesters with special respect to the influence of oligomers on the degradation rate and the potential use of these biomaterials for the development of veterinary drug delivery systems.

In **Chapter 1** the animal health care market is introduced with regard to opportunities and challenges of veterinary drug delivery systems. In the second part of this chapter biodegradable polymers used for veterinary applications are discussed with emphesis on their degradation and release properties. Current applications of biodegradable drug delivery systems in the field of reproduction control, growth promotion, vaccination and control of ectoparasites are summarized. Furthermore, perspectives and current problems in the field of biodegradable drug delivery systems are presented. This chapter concludes with the scope, aims and outline of the thesis.

Chapter 2 describes the synthesis and characterization of D,L-lactic acid oligomers. The oligomers were prepared by polycondensation without catalyst providing a series of D,L-lactic acid oligomers with narrow molecular weight distribution. The oligomers were analyzed using SEC, DSC and ^{1}H-NMR to obtain information about their physico-chemical properties as function of average chain length. The interest was focused on the determination of the critical molecular weight for water solubility of oligomers. It was found that the solubility of oligomers depends on both pH and average molecular weight. Polydisperse oligomers with $\overline{M}_n$ smaller than 830 Da are soluble in buffer at pH 7.4 whereas oligomers of $\overline{M}_n \geq 830$ Da are insoluble.

In addition the hydrolysis kinetic of oligomer degradation and the mechanism of ester cleavage was investigated in vitro. The degradation rate was dependent on the pH of incubation medium and the lowest degradation rate was found near a pH value of 4.5 independent on chain length. An Arrhenius plot was constructed for the calculation of the activation energy using an accelerated monomer release test. It was found that with increasing molecular weight of oligomer the energy of activation increased. Furthermore, it could be shown that hydrolysis under basic conditions proceeds by random ester cleavage and/ or backbiting whereas chain-end cleavage was observed under acidic conditions.

After successful synthesis and characterization of a homologous series of oligomers the effect of these low molecular weight polymers on the degradation process of

PLGA was investigated (**Chapter 3**). Hence, PLGA films containing oligomers of different molecular weight in various amounts were prepared by a solvent casting technique and the degradation was monitored by SEC, WAXD, DSC and gravimetric methods. The results demonstrated that the incorporation of oligomers clearly affects the physico-chemical properties of PLGA films. The hydrophilicity increases, whereas the glass transition temperature of the film decreases. It was found that the initial mass loss and the amount of water absorbed were functions of average molecular weight and concentration of oligomers. In contrast to results reported in literature an autocatalytic effect due to the increased number of carboxylic end groups of oligomers incorporated was not observed. Moreover, for all oligomer-containing films an extended lag phase until onset of polymer erosion was found. It could be shown that this finding was related to crystallization which occured during degradation of all films. However, the degree of crystallization was higher in films containing oligomers and was depending on oligomer concentration and oligomer molecular weight. These results lead to the assumption that oligomers cause direct or indirect crystallization during the degradation process thus reducing the degradation rate.

As discussed in the previous chapters the degradation of PLGA and PLA occurs via bulk erosion which leads often to an unfavourable release profile. Therefore in **Chapter 4** the degradation of PTA was investigated to determine if introduction of cleavable ester and ketal bonds in the polymer side chains results in improved degradation properties compared to PLGA. After preparation of PTA implants of different size by compression molding the degradation was evaluated under in vitro conditions in a comparative study using PLGA implants of same size and dimension. It was observed that the shape of PTA implants changed during degradation from a flat disc to a bloated convex device most probably due to swelling. After a definite time interval the implant shell bursts and water soluble degradation products like acetone, ethanol and tartaric acid were found in release medium. In parallel a remarkable mass loss was detected which was not observed until this time point. Possible mechanisms to explain these finding are discussed. It was also found that the implant size had a negligible impact on the degradation and erosion of PTA. Furthermore, a differentiation between surface and center as known for bulk eroding polymers was observed during the degradation of PTA implants. This leads to the conclusion that PTA is also a bulk eroding polymer as PLGA.

In **Chapter 5** the influence of the degradation behavior on drug release from PTA implants was investigated in vitro. Effects of drug loading and implant size on release profile were studied in more detail. Our hypothesis that drug release will occur in a pulsatile manner was confirmed. The release from PTA implants was characterized by an initial burst, followed by a lag-phase where no drug was released and subsequentes a second burst occured. After the second burst drug was released by zero order kinetic until the end of the study. It was found that the extend of second burst correlates with drug loading and increases with decreasing drug loading due to the reduced initial burst. Various materials were tested to modify the drug release from PTA implants. Already small amounts of excipients (10 % w/w) were sufficient either to modify the degree of second burst or to prevent the release of drug in a pulsatile manner. Based on these results PTA was identified as a promising biomaterial especially for pulsatile drug release systems.

In **Chapter 6** the in vivo release properties of buserelin implants were studied in dogs. An attempt was made to develop different levels of in vitro-in vivo correlation for biodegradable implants. Hence, PLGA implants containing different amounts of the GhRh analogon buserelin were prepared by compression molding and coated at different levels in order to modify drug release rate. By using model-independent methods such as statistical moment analysis and model-dependent methods (curve-fitting) the in vitro release profiles were compared to the in vivo performance. A level A IVIVC could be successfully established if drug release occurs mainly by diffusion through water filled pores. In addition it was found that a level B IVIVC could be developed if drug release occurs by a combination of diffusions and erosion processes. Furthermore several in vitro parameters were determined which correlate well with in vivo parameters resulting in level C IVIVC.

Perspectives

This thesis demonstrates that for understanding the degradation process of aliphatic polyesters intensive research is still required in order to improve the release characteristic from biodegradable delivery systems.

The influence of D,L-lactic acid oligomers on in vitro degradation of bulk eroding polymers was assessed in this thesis and although we demonstrated that oligomers

play an important role in this process, further research in this area is necessary to explain the complex degradation process.

In this thesis different oligomers were incorporated in PLGA films only and no autocatalytic effect was observed. It is therefore recommend to study the effect of oligomers on the degradation of massive devices such as implants in order to verify if autocatalytisis occurs in this case. Furthermore, the degradation of oligomer-containing poly(lactide-co-glycolide) films of small molecular weight were investigated only. Thus the effect of oligomers on the degradation of heigher molecular weight polyester devices should be investigated.
Another interesting approach would be to visualize the contribution and release of incorporated oligomers during the degradation process. This requires the coupling of a suitable marker at the carboxylic or hydroxylic chain end in such a way that the bond will not be cleaved under degradation conditions.
Due to the fact that the oligomers synthesized in this thesis were only used for degradation experiments further research could be focused on the potential of oligomers to modulate or to control drug release.

For the tartaric acid based polymer which has be shown its potential for pulsatile drug release first of all the polymer has to be synthesized in the desired quality and with described characteristics (M_w, T_g, particle size). Thereafter the following recommendations could be made. First, in this thesis cylindrical-shaped implants were used for in vitro degradation and release studies only. In consequence, both drug release and degradation should be evaluated from microspheres and nanospheres. It is expected that drug release will no longer occur in a pulsatile manner.
Another point that deserves attention is to investigate if a solution of PTA formes in situ a gel or implant which could be used as delivery system for drugs.

Appendices

Zusammenfassung

In Hinblick auf die Entwicklung von innovativen pharmazeutischen Veterinärprodukten wurde im Rahmen dieser Arbeit der Abbau- und Freisetztungs-mechanismus von aliphatischen Polyestern experimentell untersucht. Der erste Komplex der vorliegenden Arbeit befaßte sich mit dem Einfluß von Oligomeren auf das Abbauverhalten von PLA- und PLGA- Polymeren, im zweiten Teil wurden Polytartrate charakterisiert und ihr Potential als bioabbaubare Arzneistoffträger untersucht.

Die in der Veterinärpharmazie verwendeten klassischen und neuen bioabbaubaren Polymere wurde im ersten Kapitel kurz vorgestellt und deren Abbaumechansimus erläutert. Aspekte, die bei der Entwicklung von Tierarzneimitteln zu berücksichtigen sind, wurden dargelegt und Einblick in den Tiergesundheitsmarkt gewährt. Aktuelle Anwendungsgebiete von bioabbaubaren Arzneistoffträgersystemen für die parenterale Wirkstoffapplikation am Tier wurden dargestellt. Hierbei lag der Schwerpunkt in den Bereichen Reproduktionskontrolle, Wachstumsförderung, Immunisierung und Kontrolle von Antiparasitika. Ferner wurden Probleme und mögliche Perspektiven in der Anwendung von bioabbaubaren Polymeren zur Applikation von Wirkstoffen im Tier aufgezeigt und diskutiert.

Da der Einfluß von Oligomeren auf das Abbauverhalten und den Abbaumechanismus von Poly(D,L-laktiden) und Poly(D,L-laktid-co-glykoliden) in der Literatur widersprüchlich diskutiert wird, wurde eine Serie von D,L-Laktide Oligomeren synthetisiert und charakterisiert (Kapitel 2). Der Zusammenhang zwischen den physikalisch-chemischen Eigenschaften und dem durchschnittlichen Molekular-gewicht der Oligolaktide wurde mit Hilfe von Größenauschlußchromatographie, thermoanalytischen Verfahren sowie Kernspinspektroskopie untersucht. Dabei stand die Ermittlung des Löslichkeitsverhaltens der Oligomere in Abhängigkeit zu ihrer Kettenlänge im Vordergrund.
Die Ergebnisse zeigten, daß D,L-Laktide Oligomere mit einem Molekulargewicht von $M_n \leq 830$ Da sich bei einem pH-Wert von 7.4 in Puffer lösen, jedoch nicht in Wasser und sauren Prüfflüssigkeiten.

Da die Löslichkeit der Oligomere einen Einfluß auf die Abbaugeschwindigkeit hat, wurde in einer mechanistischen Studie die Hydrolysekinetik und der Mechanismus der Esterspaltung als Funktion der durchschnittlichen Kettenlänge untersucht.
Die Experimente zeigten den Zusammenhang zwischen Abbaugeschwindigkeit und pH-Wert des Mediums. Unabhängig vom Molekulargewicht der getesteten Oligomere wurde im pH Bereich um 4.5 die geringste Hydrolysegeschwindigkeit gemessen.
Um die Aktivierungsenergie bestimmen zu können, wurde ein Test entwickelt, mit welchem die Freisetzung des monomeren Hydrolyseproduktes (Milchsäure) untersucht werden konnte. Die Ergebnisse zeigten, daß die Aktivierungsenergie zur Spaltung der Esterbindungen mit steigendem Molekulargewicht der Oligomere ansteigt. Ferner wurde durch Variation der Hydrolysebedingungen der Mechanismus der Esterspaltung mittels ^{1}H-NMR untersucht. Es konnte gezeigt werden, daß unter basischen Bedingungen eine statistische Esterspaltung und/ oder Backbiting stattfindet, während unter sauren Bedingungen vermehrt eine Kettenendpaltung beobachtet wurde.

Um den Einfluß von D,L-Laktide Oligomeren auf die Abbaugeschwindigkeit von aliphatischen Polyestern zu untersuchen, wurden Filme aus Poly(D,L-laktide-co-glykolide) hergestellt. Oligomere unterschiedlichen Molekulargewichtes wurden in verschiedenen Konzentrationen in die PLGA Filme eingearbeitet.
Die physikalisch-chemische Charakterisierung der Filme zeigte, daß durch Einarbeitung von Oligomeren die Hydrophilie des Polymerfilmes steigt, während die Glasübergangstemperatur des Filmes sinkt. Aus den Abbauuntersuchungen ging deutlich hervor, daß sowohl der initiale Gewichtsverlust als auch die aufgenommene Wassermenge in Zusammenhang mit dem Molekulargewicht und der eingearbeiteten Oligomermenge stehen. Der in der Literatur beschriebene, auf die verhältnissmäßig hohe Anzahl an Carboxylendgruppen basierende katalytische Effekt der Oligomere, wurde im Fall der PLGA Filme nicht beobachtet. Im Gegenteil, oligomerhaltige Filme wiesen eine längere Verzögerungsphase zwischen dem initialen Massenverlust und dem Einsetzen der Erosion auf. Die Untersuchung der PLGA Filme mittels Weitwinkelröntgenbeugung zeigte, daß sowohl oligomerhaltige als auch oligomerfreie Filme im Laufe der Abbauuntersuchungen kristalline Strukturen ausbildeten. Da die Kristallinität in oligomerhaltigen Filmen höher war und zu einem früheren Zeitpunkt nachgewiesen werden konnte als in oligomerfreien Filmen, ist

anzunehmen, daß Oligomere direkt oder indirekt zur Ausbildung kristalliner Strukturen beitragen. Die Tatsache, daß kristalline Bereiche langsamer erodieren als amorphe Regionen, erklärt die unterschiedlichen Hydrolysegeschwindigkeiten innerhalb des Abbauprozesses. Die Ergebnisse weisen darauf hin, daß ein autokatalytischer Polymerabbau durch Oligomere eher unwahrscheinlich ist.

Obwohl die Freisetzung von Wirkstoffen aus PLGA Systemen aufgrund der Bulkerosion nur selten einer Kinetik nullter Ordnung folgt, ist es das wohl am häufigsten verwendete Biomaterial.
Ein auf Weinsäure basierendes Polymer, welches sowohl spaltbaren Ester- als auch Ketalbindungen enthält, ließ verbesserte Abbau- und Freisetzungseigenschaften vermuten. Da die Kettenspaltung sowohl in der Haupt- als auch in den Seitenketten stattfindet, können schneller wasserlösliche Abbauprodukten gebildet werden. Nachdem die Implantate erfolgreich durch Direktverpressung unter hohem Druck hergestellt worden waren, wurde eine vergleichende Studie zum Abbauverhalten von PTA und PLGA Polymeren durchgeführt (Kapitel 4). Um Rückschlüsse auf den Abbaumechanismus von PTA zu erhalten, wurden PTA Implantate mit unterschiedlichen Abmessungen hergestellt und erodiert. Bei den gewählten Größenverhältnissen zeigten die Abbauprofile keine Veränderung des Abbaumechanismus. Das deutet darauf hin, daß PTA Implantate nicht nach dem Mechanismus der Oberflächenerosion abgebaut werden, sondern nach dem Mechanismus der Bulkerosion. Durch Quantifizierung der Abbauprodukte konnte gezeigt werden, daß es in den PTA Implantaten zu einer Aufkonzentrierung von Weinsäure, Ethanol und Aceton kommt. Diese werden schlagartig freigesetzt, wenn das Implantat platzt und erklären den extremen Massenverlust der Implantate von diesem Zeitpunkt an. Die Quellungs- und Massenverlustprofile der PTA Implantate unterschieden sich deutlich von denen der PLGA Implantate, deßhalb wurde ein neuartiges Freisetzungsprofil erwartet.

Mittels eines Testsystemes wurde die Freisetzung des Modelpeptides Buserelin aus PTA Implantaten in vitro untersucht (Kapitel 5). Ein Vorteil des angewendeten Herstellverfahrens liegt, im Gegensatz zu bereits bekannten Verarbeitungsmethoden (Extrusion, Mikroverkapselung), in der Abwesenheit organischer Lösemittel sowie in der fehlenden Temperaturbelastung von Wirk- und Hilfsstoffen. Der Einfluß des

Beladungsgrades und der Größe des Implantates auf das Freisetzungsprofil wurde untersucht. Unabhängig von diesen Parametern wurde ein pulsatiles Freisetzungsverhalten beobachtet. Dieses war durch eine erhöhte Initialfreisetzung, gefolgt von einer Phase geringer Wirkstoffabgabe und einer sich anschließenden erneuten Phase erhöhter Wirkstofffreisetzung charakterisiert. Es konnte gezeigt werden, daß mit steigendem Beladungsgrad und abnehmendem Implantatdurchmesser der initialen Burst steigt, während die Wirkstoffmenge, die innerhalb kurzer Zeit schlagartig freigesetzt wird, abnimmt. Auf Grund dieses Zusammenhanges kann die Dosis, die pulsatil freigesetzt werden soll, angepaßt werden. Durch Variation der Implantatzusammensetzung (Zusatz hydrophiler oder lipohiler Substanzen) wurde die Möglichkeit zur Steigerung oder Reduktion der Freisetzungsgeschwindigkeit aus PTA Implantaten untersucht. Das pulsatile Freisetzungsprofil konnte durch die Zugabe von kleinen Hilfsstoffmengen (< 10 %) so geändert werden, daß der Wirkstoff nahezu konstant freigesetzt wurde. Die Untersuchungen deuten darauf hin, daß PTA Implantate als Träger für Arzneistoffe, die pulsatile freigesetzt werden sollen (Hormone, Vaccine), besonders geeignet sind.

Da basierend auf in vitro Freisetzungungen nur selten kinetische Daten vorhergesagt werden können, wurde versucht durch Anwendung der statistische Momentanalyse bzw. der mathematische Dekonvolution, in vitro Daten mit in vivo Ergebnissen zu korrelieren (Kapitel 6). Dafür wurden PLGA Implantate hergestellt, die unterschiedliche Wirkstoffmengen enthielten. Das Freisetzungsprofil wurde durch Auftragen eines Polymerfilmes modifiziert. Auf Grundlage der in vitro Untersuchungen wurden drei PLGA Implantaten unterschiedlicher Zusammensetzung ausgesucht und das Freisetzungsverhalten von Buserelin aus diesen Implantaten im Hund untersucht. Im Falle des überzogenen Implantates ergab sich eine exzellente Übereinstimmung zwischen der Freisetzung in vitro und der Absorption in vivo (Level A IVIVC), da der Wirkstoff hauptsächlich durch Porendiffusion freigesetzt wurde. Anhand der Untersuchungen konnte gezeigt werden, daß für Implantate deren Wirkstofffreisetzung sowohl durch Diffussion als auch durch Abbauvorgänge gesteuert wird, die mittlere Auflösezeit in vitro mit der mittleren Verweildauer der Substanz im Organismus korreliert (Level B IVIVC). Ferner wurden verschiedene in vitro und in vivo Größen ermittelt, für die eine Level C Korrelation erstellt werden konnte.

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